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Specialized Transduction with λ p*lac*5: Dependence on *recB*

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Genetically disabled λ p*lac*5 transducing phage derivatives were used to study the *recB* dependence of recombination during specialized transduction. The frequency of transduction was normalized to colony-forming units, and the end product of recombination was monitored by scoring for addition and substitution transductants. When a chromosomal *lac* gene was the recipient DNA substrate molecule, both the normalized transduction frequency and the proportion of addition and substitution transductants showed essentially no *recB* dependence. There was a pronounced *recB* dependence for both normalized transduction frequency and recombination end product formation when F42 *lac* was the recipient DNA substrate. *recB* appears to have no significant role in the recombination that occurs between the two *lac* regions in an addition transductant. UV irradiation of the transducing phages increased the absolute level of both addition and substitution transductants obtained with a chromosomal *lac* gene but resulted in a considerable change in the relative frequency of addition versus substitution transductants.

The role of the product of the *Escherichia coli* *recB* and *recC* genes in general recombination (4) remains somewhat obscure. Hfr conjugational crosses with *recB* recipients show significantly reduced levels of viable recombinant colony formation, whereas the initiation of recombination in that circumstance occurs at essentially normal levels (2). The frequency of bacteriophage P1-mediated generalized transduction in *recB* recipient strains is also significantly reduced (10). Studies of the dependence of the frequency of specialized transduction on *recB* have yielded conflicting results (7–9).

We have utilized genetically disabled λ p*lac*5 transducing phage derivatives (6) to compare several aspects of specialized transduction in Rec$^+$ and *recB* strains when site-specific recombination of the transducing phage DNA with the *att*λ site on the recipient cell genome is precluded by a *gal-att*λ-*bio* deletion. The *E. coli* K-12 strains and the λ p*lac*5 derivatives used are shown in Table 1. The solutions, media, and phage handling procedures were as previously described (6). Transductions were done as previously described (6), except that all transduction cultures were washed once with 56/2 before plating, and all steps were carried out at 30 to 32°C.

A series of experiments was done to examine the *recB* dependence of both the transduction frequency and the nature of the recombination end product for λ p*lac*5 transduction. λ p*lacZ*$^+$ and λ p*lacZ* were used as DNA donors, and both F42 *lac* and a chromosomal *lac* gene were used as recipient DNA substrates. As *recB* cells demonstrate reduced viability (3), colony-forming units (CFU) were determined for each transduction so that transduction levels could be normalized for cell viability. The nature of the recombination end product was examined by scoring for addition and substitution transductants. Addition transductants are those in which the entire transducing phage DNA molecule has been added to the *lac* region of a recipient cell DNA molecule by general recombination in a manner somewhat analogous to the integration of λ DNA at the genomic *att*λ site. Substitution transductants are those in which there has been an interaction of *lac* DNA sequences which yields a Lac$^+$ colony without the addition of any bacteriophage DNA sequences to the recipient DNA molecule. An addition transductant will have the λ *c*I gene present and will be resistant to infection by λ *c*71; the substitution transductant will lack λ DNA sequences and will be sensitive to λ *c*71. Grids of transductants were replica plated onto LB plates (5) spread with 10^9 λ *c*71 phage to test for addition versus substitution recombination. In all cases, each grid was tested twice. Approximately 98% of the colonies gave the same result both times; those that did not were regridded and tested twice more. We examined about 1,000 Lac$^+$ transductants from each type of cross. The results of the transduc-

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TABLE 1. *E. coli* K-12 strains

Strain	Relevant characteristics	Source, reference, comments
KL759	RDP100 (λ *c*I857 *N*am7 *N*am53 *P*am80 p*lac5* *IZ*$^+$ *Y*)	6
KL760	RDP100 (λ *c*I857 *N*am7 *N*am53 *P*am80 p*lac5* *IZ*118 *Y*)	6
RDP100	F$^-$ Δ(*lac-pro*) X111 *leu thi acrA* (?) *supE44*	6
RDP112	F$^-$ *lacZ813 lacI3* Δ(*gal-attλ-bio*) *met rpsL thi* λ$^-$	These strains are *lacZ813*(Oc) (11) derivatives of KL773 (6). The *recB21* allele was introduced from AB2470 (1) by P1 transduction.
RDP113	F$^-$ *lacZ813 lacI3* Δ(*gal-attλ-bio*) *met recB21 rpsL thi* λ$^-$	
RDP114	F42 *lacZ813 lacI3*/ Δ(*lac-pro*) X111 Δ(*gal-attλ-bio*) *met rpsL thi* λ	
RDP115	F42 *lacZ813 lacI3*/ Δ(*lac-pro*) X111 Δ(*gal-attλ-bio*) *met recB21 rpsL thi* λ$^-$	

tion frequency and recombination end product analysis are shown in Table 2.

It can be seen that the number of Lac$^+$ transductants per CFU was only twofold higher for a Rec$^+$ than for a *recB* strain for transduction with either λ p*lacZ*$^+$ or λ p*lacZ* in the chromosomal *lac* recipient case. The ratio of addition to substitution transductants was also essentially identical for either phage in a Rec$^+$ or a *recB* strain when a chromosomal *lac* gene was the recipient DNA substrate. This strongly implies that the *recB* gene product has very little, if any, role in recombination between λ p*lac5* and a chromosomal *lac* gene. It can also be seen that there is a significantly higher percentage of addition transductants for F42 *lac* × λ p*lacZ* than for chromosomal *lac* times λ p*lacZ* in Rec$^+$ strains. This result demonstrates that the previously reported enhanced recombination between F42 *lac* and λ p*lac* (6, 7) involves a variation in the recombination mechanism from the chromosomal *lac* times λ p*lac* case. The correspondence of both Lac$^+$ transductants per CFU and the percentage of addition transductants for either F42 *lac* or chromosomal *lac* in a *recB* strain also demonstrates that the *recB* gene product is absolutely required for the mechanism that produces the enhanced recombination between F42 *lac* and λ p*lac* in a Rec$^+$ strain.

Addition transductants obtained with λ p*lac5* represent a situation in which the recipient cell contains two copies of the *lac* genes surrounding the added λ DNA sequences. This configuration allows for recombination between two copies of *lac* genes to generate various types of derivatives. To examine the generation of such derivatives, 10 addition transductants from each type of cross were streaked on an LB plate at 30°C, and 100 colonies from each streak were gridded on another LB plate. The patches on these grids were tested for Lac$^+$ and Lac$^-$ on MacConkey agar plates and for the presence of λ DNA sequences (specifically the λ *c*I gene) by replica plating on LB plates spread with λ *c*71. The results of this analysis are shown in Table 3.

The Lac$^+$ λ *c*71^r colonies obtained in these experiments were presumably unchanged addition transductants, whereas the other colony types were derivatives that resulted from some type of recombination event between the tandem *lac* regions. In all situations that involved a chromosomal *lac* gene, the addition transductants were very stable. The rate of derivative

TABLE 2. Effect of *recB* on λ p*lac5* transduction frequency and recombination end product[a]

Recipient strain	Phage	Lac$^+$/ml	CFU/ml	Lac$^+$/CFU	Addition transductants (λ *c*71^r)
F$^-$ *lacZ recB*$^+$	λ p*lacZ*$^+$	6.3×10^3	2.5×10^8	2.5×10^{-5}	638/1,069 (64.2)
(RDP112)	λ p*lacZ*$^-$	1.3×10^3	2.3×10^8	5.6×10^{-6}	396/1,350 (29.3)
F$^-$ *lacZ recB21*	λ p*lacZ*$^+$	6.7×10^2	6.3×10^7	1.1×10^{-5}	685/1,057 (64.8)
(RDP113)	λ p*lacZ*$^-$	2.1×10^2	6.9×10^7	3.0×10^{-6}	336/1,066 (31.5)
F42 *lacZ*/Δ(*lac*)*recB*$^+$	λ p*lacZ*$^+$	7.1×10^4	2.2×10^8	3.2×10^{-4}	723/1,061 (68.1)
(RDP114)	λ p*lacZ*$^-$	4.9×10^4	2.4×10^8	2.0×10^{-4}	586/1,050 (55.8)
F42 *lacZ*/Δ(*lac*)*recB21*	λ p*lacZ*$^+$	4.2×10^2	6.5×10^7	6.5×10^{-6}	780/1,241 (62.9)
(RDP115)	λ p*lacZ*$^-$	1.5×10^2	5.5×10^7	2.8×10^{-6}	272/966 (28.2)

[a] Transductions were carried out using λ *c*I857 *N*am7 *N*am53 *P*am80 p*lacZ*$^+$ from KL759 and λ *c*I857 *N*am7 *N*am53 *P*am80 p*lacZ118* from KL760 at a multiplicity of infection of 0.1. CFU were determined by dilution platings on LB plates. The values shown for Lac$^+$/CFU are averages from four to six experiments for each transduction. There were fewer than three Lac$^+$ colonies per ml in uninfected control cultures for all of the strains used. The transductants were tested for addition versus substitution as described in the text. The numbers for each cross are given as addition transductants (λ *c*71^r) per total transductants tested, with the percentage of addition transductants shown in parentheses.

TABLE 3. Derivatives from addition transductants[a]

Recipient strain	Phage	No. of colonies obtained			
		Lac$^+$ λ *c71*^{r}	Lac$^+$ λ *c71*^{s}	Lac$^-$ λ *c71*^{r}	Lac$^-$ λ *c71*^{s}
F$^-$ *lac recB*$^+$	λ *placZ*$^+$	963	12	1	24
(RDP112)	λ *placZ*	984	2	4	10
F$^-$ *lac recB21*	λ *placZ*$^+$	952	17	1	30
(RDP113)	λ *placZ*	898	52	47	3
F42 *lac*/Δ(*lac*)*recB*$^+$	λ *placZ*$^+$	819	67	11	103
(RDP114)	λ *placZ*	706	95	49	150
F42 *lac*/Δ(*lac*)*recB21*	λ *placZ*$^+$	655	17	13	315
(RDP115)	λ *placZ*	803	16	18	163

[a] Ten addition transductants from each type of cross were streaked on LB plates, and 100 colonies from each streak were analyzed for their Lac$^\pm$ phenotype and resistance or sensitivity to λ *c71* as described in the text.

formation and the nature of the derivatives formed differed little in the Rec$^+$ as opposed to the *recB* strain. The difference between RDP112 and RDP113 in Table 3 is actually less significant than it might appear, as the changed derivatives formed from the addition transductants of RDP113 with λ *placZ* resulted largely from a "jackpot" obtained with a single isolate. This observation implies that *recB* plays little or no role in the recombination between the two *lac* regions in this situation, as we would otherwise expect a higher level of recombination-mediated derivative production in a Rec$^+$ strain than in a *recB* strain. The addition transductants obtained with F42 *lac* were somewhat less stable than those in the chromosomal *lac* case, but they showed approximately the same level of recombination events between the two *lac* gene regions in a Rec$^+$ or a *recB* strain.

Previous studies with λ *plac5* transduction examined the levels of initiation of recombination by measuring the transcribable intermediate (7). It was found that UV-irradiated λ *plac5* and a chromosomal *lac* gene give approximately the same level of transcribable intermediate as F42 *lac* times non-UV-irradiated λ *plac5*. This UV irradiation-mediated stimulation of recombination initiation between λ *plac5* and a chromosomal *lac* gene occurs to essentially equal extents in Rec$^+$ and *recB* strains (7). We used our genetically disabled λ *plac5* transducing phages to look at the effect of UV irradiation on the production of viable transductant colonies in Rec$^+$ versus *recB* strains. We also examined the effect on addition versus substitution transduction of UV irradiation of the λ *plac5* phages when a chromosomal *lac* gene is the recipient DNA substrate in a Rec$^+$ strain.

Transducing phage stocks in λ buffer (6) were irradiated with 90 J of 254-nm light per m^2 from a germicidal lamp. Rec$^+$ and *recB* strains containing either F42 *lac* or a chromosomal *lac* gene were transduced with irradiated and non-irradiated stocks of λ *placZ*$^+$ and λ *placZ*. The results are presented in Table 4 in terms of Lac$^+$/CFU. Transductants obtained with RDP112 and UV-irradiated λ *plac5* derivatives were tested for addition versus substitution transduction as described above; the results are shown in Table 4.

UV irradiation of the transducing phages produced a comparable stimulation of viable transductants in a Rec$^+$ or a *recB* strain for a chromosomal *lac* recipient and for F42 *lac* in a *recB* strain (Table 4). This result shows that the *recB* gene product is not required for the higher levels of recombination seen with the UV-irradiated transducing phage and again demonstrates the lack of a role for the *recB* gene product in the recombination between λ *plac5* and a cellular *lac* gene, with the exception of its role in the enhanced recombination between F42 *lac* and λ *plac5*. The results with RDP112 also show that the large increase in transduction frequency is accompanied by a dramatic reduction in the percentage of addition transductants with either λ *placZ*$^+$ or λ *placZ*. This change in the percentage of addition transductants represents an increase in the absolute frequency of both addition and substitution transduction, with a much greater relative increase in the frequency of substitution transductants. The contrast in the percentages of addition transductants obtained with UV-irradiated λ *plac5* times chromosomal *lac* versus non-UV-irradiated λ *plac5* and F42 *lac* indicates that the mechanism of recombination stimulation from UV treatment of the transducing phage differs markedly from the *recB*-dependent mechanism that normally produces enhanced levels of recombination when F42 *lac* is the recipient DNA molecule. Although we have not tested for the effect of UV irradiation of the transducing phage on the recombination end product for all possible cases, the effect for a chromosomal *lac* gene in a Rec$^+$ strain (RDP112) is striking. The results in this case show that UV irradiation does not merely increase the frequency of recombination events but also results in a perturbation of the recombination mechanism that is reflected in the end product.

Although some previous studies have indicated that there is little *recB* dependence for specialized transduction of a chromosomal marker (7, 8), the present study has strengthened that conclusion by normalizing transduction to CFU and by using minus times minus allele crosses with genetically disabled phages that result in no host cell killing. This work also further clarifies the role of the *recB* gene product in the enhanced recombination between F42 *lac* and λ

TABLE 4. Effect of UV irradiation of transducing phage on transduction[a]

Strain	Phage	UV irradiation	Lac$^+$/CFU	Addition transductants (λ $c71^r$)
F$^-$ *lac recB*$^+$ (RDP112)	λ *placZ*$^+$	−	2.3×10^{-5}	686/1,069 (64.2)
	λ *placZ*$^+$	+	1.9×10^{-3}	74/500 (14.8)
	λ *placZ*	−	6.5×10^{-6}	396/1,350 (29.3)
	λ *placZ*	+	1.1×10^{-3}	22/500 (4.4)
F$^-$ *lac recB21* (RDP113)	λ *placZ*$^+$	−	7.0×10^{-6}	685/1,057 (64.8)
	λ *placZ*$^+$	+	4.8×10^{-4}	ND[b]
	λ *placZ*	−	3.5×10^{-6}	336/1,066 (31.5)
	λ *placZ*	+	2.3×10^{-4}	ND
F42 *lac*/Δ(*lac*)*recB*$^+$ (RDP114)	λ *placZ*$^+$	−	1.9×10^{-4}	723/1,061 (68.1)
	λ *placZ*$^+$	+	8.0×10^{-4}	ND
	λ *placZ*	−	1.4×10^{-4}	568/1,050 (55.8)
	λ *placZ*	+	3.7×10^{-4}	ND
F42 *lac*/Δ(*lac*)*recB21* (RDP115)	λ *placZ*$^+$	−	6.0×10^{-6}	780/1,241 (62.9)
	λ *placZ*$^+$	+	4.8×10^{-4}	ND
	λ *placZ*	−	6.7×10^{-6}	272/966 (28.2)
	λ *placZ*	+	3.7×10^{-4}	ND

[a] Phage stocks in λ buffer were irradiated with 90 J of 254-nm light per m^2 from a germicidal lamp, which resulted in approximately a threefold reduction in phage titer. Transductions were done at a multiplicity of infection of 0.1 based on the titer of non-irradiated phage, and platings were done on LB plates to determine CFU in the transduction cultures. Transductants obtained with RDP112 and UV-irradiated phage were tested for addition (λ $c71^r$) or substitution (λ $c71^s$) as described in the text. The values for addition transductants with non-irradiated phage were taken from Table 2; the percentage of addition transductants is shown in parentheses.

[b] ND, Not done.

plac5 (6, 7) by demonstrating that the normalized transduction frequency and the percentages of addition and substitution transductants are essentially the same for both F42 *lac* and a chromosomal *lac* gene in a *recB* recipient cell.

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LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Birge, E. A., and K. B. Low.** 1974. Detection of transcribable recombination products following conjugation in Rec$^+$, RecB$^-$, and RecC$^-$ strains of *Escherichia coli* K-12. J. Mol. Biol. **83**:447–457.
3. **Capaldo, F. N., G. Ramsey, and S. D. Barbour.** 1974. Analysis of the growth of recombination-deficient strains of *Escherichia coli* K-12. J. Bacteriol. **118**:242–249.
4. **Low, K. B., and R. D. Porter.** 1978. Modes of gene transfer and recombination in bacteria. Annu. Rev. Genet. **12**:249–287.
5. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Porter, R. D., M. W. Lark, and K. B. Low.** 1981. Specialized transduction with λ *plac5*: dependence on *recA* and on configuration of *lac* and *att*λ. J. Virol. **38**:497–503.
7. **Porter, R. D., T. McLaughlin, and B. Low.** 1978. Transduction versus "conjuduction": evidence for multiple roles for exonuclease V in genetic recombination in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **43**:1043–1047.
8. **Wackernagel, W., and C. M. Radding.** 1974. Transformation and transduction of *Escherichia coli*: the nature of recombinants formed by Rec, RecF, and λRed, p. 111–122. *In* R. F. Grell (ed.), Mechanisms in recombination. Plenum Publishing Corp., New York.
9. **Weisberg, R. A., and N. Sternberg.** 1974. Transduction of *recB*$^-$ hosts is promoted by λ*red*$^+$ function, p. 107–109. *In* R. F. Grell (ed.), Mechanisms in recombination. Plenum Publishing Corp., New York.
10. **Willets, N. S., and D. W. Mount.** 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations cotransducible with *thyA*. J. Bacteriol. **100**:923–934.
11. **Zipser, D.** 1967. Orientation of nonsense codons on the genetic map of the Lac operon. Science **157**:1176–1177.