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Specialized Transduction with λplac5: Dependence on *recA* and on Configuration of *lac* and *att*λ

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The construction of λ*plac5* transducing phages carrying various *lacZ* alleles is described. Genetically disabled (N^- N^- P^-) λ*plac* transducing phages were used to study the dependence of specialized transduction on host RecA function and on the location of the *lacZ* gene in the recipient strain. In the absence of site-specific recombination at *att*λ, transduction was completely dependent on host RecA function. Regardless of the configuration of *att*λ, λ*plac* transducing phages recombined at a 20- to 50-fold higher frequency with F42*lac* than with a *lac* gene located in the cellular chromosome. Deletion mutants of *lacZ* in the recipient strain were used to show that the probability of *lac* recombination resulting from λ*plac* infection is apparently proportional to the amount of homology between the parental *lacZ* genes.

Specialized transduction is a mode of gene transfer (2, 9) which has been used infrequently in recombination studies. We recently described a system for the study of recombination kinetics that employs λ*plac5* transduction (13), and here we report a more detailed characterization of the λ*plac5* transduction system. The isolation and characterization of λ*plac* derivatives containing specific *lacZ* alleles is described. We also report the construction of genetically disabled λ*plac* transducing phages and their use to examine the dependence of transduction on: (i) the host *recA* gene, (ii) the location of the *lacZ* allele in the recipient strain, and (iii) the configuration of the *att*λ site in the genome of the recipient cell. The randomness with which recombination can occur along the *lacZ* gene in specialized transduction is evaluated.

MATERIALS AND METHODS

Bacterial strains. The *Escherichia coli* K-12 derivatives used in this study are listed in Table 1. The complete pedigree of all strains is available on request.

Bacteriophage. The bacteriophage lambda and λ*plac5* derivatives used in this study were prepared from the lysogenic strains shown in Table 1.

Media. LB medium (11) was used for liquid culture medium and agar plates whenever a rich medium was required. Modified minimal medium 56 and its supplementation to produce selective media have been described (8). Half-strength modified minimal medium 56 (56/2) was used for the dilution and washing of cells. Dilutions of phage stocks were made in λ buffer (0.006 M Tris, pH 7.2, 0.01 M $MgSO_4$, and 0.005% Difco gelatin). Top agar was either 0.6% or 0.8% Difco agar in deionized water.

Lysate production. Lysates of bacteriophage carrying the λcI857 allele were prepared by heat induction of an appropriate lysogenic bacterial strain. Lysates of λcI$^+$ or λ*i*21 phage were prepared by induction with UV irradiation of appropriate lysogenic bacterial strains.

Preparation of lysogens. Lysogens for particular bacteriophage derivatives were prepared by spotting a lysate onto or stabbing a plaque into a lawn of the desired host strain and then screening colonies obtained from the area of clearing for the presence of the bacteriophage as a prophage.

Plating of bacteriophage for plaques. Phage stocks to be plated for plaques were diluted in λ buffer and plated on an appropriate indicator strain which had been grown to 10^9 cells per ml in LB medium supplemented to 0.2% maltose and 0.01 M $MgSO_4$.

Testing plaques for the Lac character of the phage. Platings of λ*plac* on Δ(*lac-pro*) strains on LB plates were used to assess the *lacZ*$^+$ or *lacZ* character of individual plaques or populations of plaques. Phage platings that had been incubated for 10 to 16 h at 37°C were flooded with 0.013 M *o*-nitrophenol-β-D-galactopyranoside (ONPG) in 0.25 M potassium phosphate buffer (pH 7.25). Plaques from λ*placZ*$^+$ phage gave a strong yellow color in less than 1 min at room temperature. Plaques from λ*vir* or λ*placZ* gave no yellow color after more than 2 h of incubation at room temperature. Platings containing both λ*placZ*$^+$ and λ*placZ* had to be evaluated within approximately 5 min because the yellow color from λ*placZ*$^+$ plaques diffused rapidly across the plate. Stabs of λ*placZ* plaques from mixed populations into a fresh lawn of cells demonstrated no yellow color after prolonged room-temperature incubation with ONPG. λ*placZ*

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

Strain	Relevant characteristics	Source, reference, comments
KL528	F$^-$Δ(*lac-pro*) *supF trp pyrF his rpsL thi* λ$^-$	CSH28 (11) cured of F′
KL550	RDP 100 (λcI857 *Sam7 plac5* I^- Z^+ Y^-)	Phage from CSH66 (11)
KL551	RDP 100 (λcI857 *Sam7 plac5* I^- *Z118*Y^-)	See text
KL754	F$^-$Δ(*lac-pro*) X111 *supG rpsL thi* λ$^-$	Derived from BW113 (8) × X7151 (from J. Miller) and spontaneous Str^r [a]
KL755	RDP 100 (λ*i21 Pam80*)	Phage from I. Herskowitz
KL756	C600 (λ*cI857ind Nam7Nam53*)	Phage from C. M. Radding
KL757	RDP 100 (λcI857 *Sam7Nam7Nam53 plac5* I^- Z^+ Y^-)	See text
KL758	RDP 100 (λcI857 *Sam7Nam7Nam53 plac5* I^- *Z118*Y^-)	See text
KL759	RDP 100 (λcI857 *Nam7Nam53Pam80 plac5* I^- Z^+ Y^-)	See text
KL760	RDP 100 (λcI857 *Nam7Nam53Pam80 plac5* I^- *Z118*Y^-)	See text
KL761	F$^-$*lacZ813 lacI3 pro met trp rpsL thi* λ$^-$	All of these strains (KL761–KL772) are *lacZ813*-ochre (21) derivatives of KL318 (1). In each case the final Rec$^+$/*recA1* pair was made by a mating with MA1079, which is a *serA* derivative (W. K. Maas) of KL16-99 (7).
KL762	KL761 but *recA1*	
KL763	F$^-$*lacZ813 lacI3 met* Δ(*gal-att*λ*-bio*)*rpsL thi* λ$^-$	
KL764	KL763 but *recA1*	
KL765	F$^-$*lacZ813 lacI3 pro met his trp rpsL thi* (λ*ind*)	
KL766	KL765 but *recA1*	
KL767	F42*lacZ813 lacI3*/Δ(*lac-pro*) *met trp rpsL thi* λ$^-$	
KL768	KL767 but *recA1*	
KL769	F42*lacZ813 lacI3*/KL773	
KL770	KL769 but *recA1*	
KL771	F42*lacZ813 lacI3*/Δ(*lac-pro*) *met his trp rpsL thi* (λ*ind*)	
KL772	KL771 but *recA1*	
KL773	F$^-$Δ(*lac-pro*)Δ(*gal-att*λ*-bio*) *met rpsL thi* λ$^-$	From KL320 (1)
RDP 100	F$^-$Δ(*lac-pro*) X111 *leu thi acrA* (?) *supE44*	C600 derivatives (RDP 100, RDP 101) with Δ(*lac-pro*) X111 derived from PK191 (8).
RDP 101	F$^-$Δ(*lac-pro*) X111 *leu thi supE44*	
RDP 103	F42*lacZ118 lacI3*(KL773)	
RDP 104	F$^-$*lacZ118 lacI3* Δ(*gal-att*λ*-bio*) *met rpsL thi*	From KL334 (1) × KL773
RDP 105	F128*lacZ*Δ*H111 proA*$^+$,*B*$^+$/KL773	From CHS14 × KL773
RDP 106	F128*lacZ*Δ*H119 proA*$^+$,*B*$^+$/KL773	From CSH15 × KL773
RDP 107	F128*lacZ*Δ*H114 proA*$^+$,*B*$^+$/KL773	From CSH16 × KL773
RDP 108	F128*lacZ*Δ*H145 proA*$^+$,*B*$^+$/KL773	From CSH17 × KL773
RDP 109	F128*lacZ*Δ*H125 proA*$^+$,*B*$^+$/KL773	From CSH18 × KL773
RDP 110	F128*lacZ*Δ*H138 proA*$^+$,*B*$^+$/KL773	From CSH19 × KL773
RDP 111	F128*lacZ*Δ*H220 proA*$^+$,*B*$^+$/KL773	From CSH20 × KL773

[a] Str^r, Streptomycin resistance.

phage containing nonsense mutations in the *lacZ* gene could be readily detected by using an appropriate suppressor strain for plating and looking for yellow color with ONPG.

Isolation of λ*plac* derivatives containing particular *lacZ* alleles. Strains containing a deletion of the *gal-att*λ*-bio* region of the chromosome and possessing the desired *lacZ* allele on the chromosome or on an F*lac* were lysogenized with λ*placZ*$^+$ as described above. These lysogens were used to prepare lysates, and the resulting phage were plated on an appropriate strain for testing the Lac character of the plaques with ONPG flooding. Plaques that were Lac$^-$ were stabbed into fresh lawns for retesting, and lysogens were prepared for those that tested Lac$^-$ twice.

Lysates from the newly obtained λ*placZ* lysogens were tested with ONPG after plating on appropriate suppressor and nonsuppressor strains when *lacZ* nonsense mutations were involved. Presumptive λ*placZ* phage were also tested by transducing strains containing the same *lacZ* allele as well as strains containing other *lacZ* alleles (see below).

Phage crosses. Phage crosses were done by infecting a suitable host strain at 2×10^8 cells per ml in LB medium supplemented to 0.2% maltose and 0.01 M $MgSO_4$ with both parental phages at a multiplicity of

infection (MOI) of 5. The plaques from these lysates were tested as described above, and the desired recombinant phages were recovered from stabs as lysogens.

Production of disabled λplacZ transducing agents. The original λ*placZ* isolates are capable of lysing cells that they infect, and this complicates transduction studies when potential transductants are lost. To avoid the loss of potential transductants by phage-mediated cell death, disabled versions of λ*plac5* were made. The *Nam7Nam53* markers were introduced by crossing λ*placZ*$^+$ with λcI857 *ind Nam7Nam53* and scoring for *lacZ*$^+$-containing phage that required an amber suppressor in the host strain for growth. The resulting λ*placZ*$^+$ *Nam7Nam53* phage was then crossed with λ*placZ118* to give a *lacZ* phage that required an amber suppressor in the host strain for growth. λ*placZ*$^+$ *Nam7Nam53* was then crossed with λ*i*21 *Pam80* to yield a *lacZ*$^+$-containing phage which specifically required *supE* for growth (*Nam7Nam53* is suppressed by either *supE* or *supF*, whereas *Pam80* is suppressed by *supE* but not *supF*) and which failed to complement either a λ *Nam7Nam53* phage or the λ*i*21 *Pam80* phage when the two types were spotted together on a *sup*$^+$ strain. The resulting λ*placZ*$^+$ *Nam7Nam53Pam80* phage was then crossed with λ*placZ118 Nam7Nam53* to obtain λ*placZ118 Nam7-Nam53Pam80*. These transducing phages containing both *Nam7Nam53* and *Pam80* alleles produced no reduction in viable cell number when they were used to infect *sup*$^+$ strains, and they were used for the transduction experiments to be described below.

Transduction procedure. The recipient cells were grown to 2×10^8 cells per ml in LB medium supplemented to 0.2% maltose and 0.01 M $MgSO_4$ at 37°C. The transducing phage preparation was added at the desired MOI as indicated for individual experiments, and incubation was continued at 37°C for 30 min. Dilutions were made with 56/2 medium, and platings were done on minimal lactose selection plates which were incubated at the desired temperature for the development of colonies.

Chemicals and media. Tryptone, yeast extract, and agar were obtained from Difco. OPNG and sugars were obtained from Sigma Chemical Co. All other chemicals were reagent grade.

RESULTS

Isolation and characterization of λplac5 derivatives carrying *lacZ* alleles. Our efforts to construct λ*plac5* derivatives containing *lacZ* alleles have been concentrated on *lacZ118* and *lacZ813*-ochre because of their suitability for recombination studies (13). The methodology, which is similar to the techniques used by others (5, 10, 14), is also suitable for other *lacZ* alleles, however, and we have also employed it to obtain a derivative of λ*plac5* carrying the *lacZ36* allele. Strains carrying a deletion of the *gal-att*λ*-bio* region of the chromosome and one of these *lacZ* alleles either on the chromosome or on F42*lac* were lysogenized with λ*plac5* containing *lacZ*$^+$. Such lysogens were characterized as Lac$^+$ and temperature sensitive at 42°C since the phage carries the λcI857 marker. The lysogens obtained segregated Lac$^-$, temperature-resistant colonies at a variable rate (1 to 50%). Phage lysates were prepared and plated on KL528, and the plaques were scored for *lacZ*$^+$ *or lacZ* by flooding with ONPG as described in Materials and Methods. The plaques containing the original *lacZ*$^+$ version of λ*plac5* developed yellow color very rapidly, whereas those that had picked up the *lacZ* allele showed no yellow color.

In the cases of *lacZ118* and *lacZ813*-ochre, we examined 1,000 plaques from each of at least 10 independent isolates containing the *lacZ* allele in the chromosome and 10 independent isolates containing the *lacZ* allele on F42*lac*. The results are shown in Table 2.

Characterization of λplacZ isolates. Representative plaques that appeared Lac$^-$ when plated on KL528 and flooded with ONPG were transferred to fresh lawns of KL754 and KL528 with sterile toothpicks and retested with ONPG after 12 h of incubation at 37°C. Both *lacZ118* and *lacZ813*-ochre are suppressible by the *supG* allele of KL754, and the stabs of phage carrying *lacZ118* or *lacZ813*-ochre produced yellow color within 5 min of flooding with ONPG. This result serves to demonstrate the sensitivity of the ONPG flooding method for detecting β-galactosidase since the efficiency of suppression of *lacZ813*-ochre by *supG* is only about 1% (R. D. Porter, unpublished data).

Lysogens of selected λ*placZ118* and λ*placZ813*-ochre isolates were prepared as described in Materials and Methods. Lysates were then prepared and plated on both KL528 (*supF*) and KL754 (*supG*) for testing with ONPG. With both of these phage isolates, the plaques gave no

TABLE 2. *Lac$^-$ plaques obtained from lysates of λcI857 Sam7 placZ$^+$ lysogens of various strains*[a]

Lysogen isolate	Lac$^-$/total plaques on strain:			
	KL769	KL763	RDP 103	RDP 104
1	28/1,019	40/1,186	174/1,896	86/1,728
2	108/1,118	18/1,091	24/1,226	13/1,502
3	30/1,192	22/1,553	5/1,716	4/1,073
4	20/1,758	11/1,336	241/1,236	0/1,116
5	51/1,893	10/1,010	125/1,013	16/1,072
6	49/1,055	10/1,788	45/1,067	9/1,054
7	29/1,772	13/1,102	175/1,096	23/1,178
8	32/1,510	0/1,674	27/1,095	56/1,631
9	44/1,044	18/1,046	628/1,320	15/2,243
10	131/1,166	20/1,171	59/1,956	2/1,050
11	34/1,152		31/1,653	

[a] λcI857 *Sam7 placZ*$^+$ lysogens of the indicated strains were prepared using phage prepared from KL550. Lysates from each lysogen were plated on KL528, and the platings were flooded with ONPG after approximately 12 h of incubation at 37°C. The numbers given represent Lac$^-$ plaques/total plaques.

yellow color when KL528 platings were flooded with ONPG, and the plaques yielded a uniform level of yellow color when KL754 platings were tested. These lysates were also used in transduction experiments with recipient strains carrying the same *lacZ* allele as the transducing phage and recipient strains carrying the other *lacZ* allele. In the selfing experiments where both the transducing phage and the recipient strain carried the same *lacZ* allele, Lac$^+$ colonies were observed at only the same level as control platings for spontaneous revertants (see Table 3). When the transducing phage and the recipient strain carried the two different *lacZ* alleles, Lac$^+$ transductants were observed at levels several orders of magnitude greater than spontaneous reversion. The details of transduction experiments are given below.

Dependence of λ*plac5* transduction on *recA*, *att*λ, and the location of the recipient *lacZ* gene. We have previously reported that λ*plac5* transducing phage recombine more efficiently with F42*lac* or F128*lac* than with a *lacZ* gene located on the chromosome, and that *lacZ* × *lacZ* recombination is totally dependent on RecA function in the transduction system (13). In those studies, nondisabled λ*plac* transducing phages were used and a resident λ*ind* prophage in the recipient strain was used to control the lytic functions of the transducing phage. We have now used the disabled transducing phage to make a more detailed analysis of the parameters affecting recombination frequency in λ*plac* transduction.

A series of strains was constructed to test the importance of: (i) F*lacZ813*-ochre/Δ*lac* versus F$^-$*lacZ813*-ochre as the recipient DNA structure, (ii) *recA*$^+$ or *recA*, and (iii) a normal *att*λ site as opposed to a λ*ind* prophage or a *gal-att*λ-*bio* deletion. Strains containing all combinations of these parameters were transduced with λ*placZ118 Nam7Nam53Pam80* and λ*placZ*$^+$ *Nam7Nam53Pam80* at an MOI of 2, and platings on minimal lactose selection plates were incubated at 37°C. The results of these transduction experiments are shown in Table 3. It can be seen that *lacZ* × *lacZ* recombination is totally dependent on *recA*$^+$, since only background reversion levels of Lac$^+$ colonies were present when *recA* strains were used as recipi-

TABLE 3. *Dependence of λplac transduction on several parameters*[a]

Strain	Recipient Lac gene	RecA	λ attachment site	Expt. no.	Lac$^+$ colonies per ml[b]		
					λ*placZ*$^+$	λ*placZ118*	No phage[c]
KL761	Chromosome	+	*att*λ	1	2.4×10^5	6.5×10^4	8
				2	6.7×10^4	3.8×10^4	12
KL762	Chromosome	*recA1*	*att*λ	1	8.3×10^4	7[c]	5
				2	3.0×10^4	3[c]	3
KL763	Chromosome	+	Δ(*gal-att*λ-*bio*)	1	4.2×10^4	3.7×10^4	6
				2	3.5×10^4	4.1×10^4	5
KL764	Chromosome	*recA1*	Δ(*gal-att*λ-*bio*)	1	0[d]	1[c]	3
				2	4[c]	2[c]	3
KL765	Chromosome	+	λ*ind*	1	4.2×10^5	5.5×10^4	2
				2	6.6×10^5	7.8×10^4	3
KL766	Chromosome	*recA1*	λ*ind*	1	7.1×10^4	3[c]	2
				2	9.4×10^4	3[c]	2
KL767	F*lac*	+	*att*λ	1	2.0×10^6	5.4×10^6	9
				2	1.9×10^6	6.0×10^6	16
KL768	F*lac*	*recA1*	*att*λ	1	7.1×10^4	2[c]	4
				2	4.0×10^4	1[c]	7
KL769	F*lac*	+	Δ(*gal-att*λ-*bio*)	1	9.8×10^5	2.5×10^6	13
				2	6.5×10^5	2.2×10^6	3
KL770	F*lac*	*recA1*	Δ(*gal-att*λ-*bio*)	1	0[d]	3[c]	9
				2	0[d]	2[c]	4
KL771	F*lac*	+	λ*ind*	1	2.0×10^6	3.8×10^6	13
				2	2.3×10^6	1.2×10^6	6
KL772	F*lac*	*recAl*	λ*ind*	1	2.1×10^4	3[c]	4
				2	1.1×10^4	5[c]	4

[a] The indicated strains were transduced with λcI857 *Nam7Nam53Pam80 placZ*$^+$ from KL759 and λcI857 *Nam7Nam53Pam80 placZ118* from KL760 as described in the text at an MOI of 2. The minimal lactose selection plates were incubated at 37°C for 48 to 72 h before colonies were counted. All recipient strains carry *lacZ813*-ochre (21).

[b] Except as indicated.

[c] Per 0.2 ml.

[d] Per 0.1 ml.

ents in transduction. When λ*placZ*$^+$ transducing phage were used with *recA* recipients that contain a normal *att*λ or a λ*ind* prophage, significant levels of Lac$^+$ transductants were seen. These Lac$^+$ transductants did not appear in a *recA* cell containing the *gal-att*λ*-bio* deletion, indicating that this type of transduction is totally dependent on site-specific recombination of the transducing phage at *att*λ (3, 4).

There was a striking effect of location of the recipient *lac* region on Lac$^+$ transduction frequency. A 20- to 50-fold-higher transduction frequency for F*lac*-carrying strains was observed, compared to F$^-$*lac*, in the cases with *att*λ or Δ(*gal-att*λ*-bio*). In the λ*ind* strains this differential holds for transduction with λ*placZ118*, but the effect appears smaller (only fourfold) with λ*placZ*$^+$. However, this difference with λ*placZ*$^+$ and λ*placZ118* hinges on the rate observed in *recA*$^+$ cells containing the resident prophage where Lac$^+$ colonies can arise from general recombination between λ*placZ*$^+$ and the resident prophage but not from recombination between λ*placZ118* and the resident prophage. This enhanced recombination of λ*plac* with F*lac* is not due to any functions supplied in *trans* by the F factor since the presence of a non-*lac*-containing F factor (either in the autonomous state as F15*thy* or integrated into the genome as a Cavalli Hfr) had no effect on the level of recombination observed between λ*plac* and a chromosomal *lac* allele (13).

Transduction of *lacZ* deletions with λ*placZ*$^+$. λ*plac5* carries the entire *lacZ* gene and part of the *lacI* gene as well as part of the *lacY* gene. The *lacZ* gene makes up the bulk of the *lac* material carried by λ*plac5* since it is about 3,700 base pairs out of about 4,200 base pairs of total Lac DNA (15). To rule out the possibility of recombinational hot spots in our transduction assay, we wanted to determine whether or not recombination can occur fairly randomly across the Lac region carried by the transducing phage. To test this possibility, transduction experiments were done with recipients that carried deletions starting at a point beyond the *lacI* gene and extending various distances into the *lacZ* gene (11). The *lacZ* deletions were carried on F128*lac-pro* in a Δ(*lac-pro*) strain containing a deletion of the *gal-att*λ*-bio* region. These strains were transduced with λ*placZ*$^+$ *Nam7Nam53Pam80* at an MOI of 2, and the lactose selection plates were incubated at 37°C. The results of these experiments are shown in Table 4. The level of Lac$^+$ transductants obtained is roughly proportional to the amount of *lacZ* material remaining in the recipient F128 episomes and indicates that recombination is reasonably random across the Lac gene material carried by λ*plac5*.

DISCUSSION

We have described in detail our version of a technique for transferring and *lacZ* allele onto λ*plac5* by using *att*λ deletion strains containing the desired *lacZ* allele. Although the frequency of λ*placZ* phage obtained varies considerably from isolate to isolate (Table 2), the method works well for the two *lacZ* alleles tested extensively and also works for other *lacZ* alleles (unpublished data). For reasons that are not entirely clear, the chances of obtaining the desired *lacZ* allele on λ*plac* is three- to fivefold better if the *lacZ* allele is located on F42*lac* rather than on the cellular genome (see Table 2). This may be related, however, to the finding that λ*plac5* initially recombines more readily with F42*lac* than with a chromosomal *lac* gene (see Table 3 and reference 13). The mechanism for generating λ*placZ* phage presumably involves recombination between the two tandem *lac* regions surrounding the prophage in an addition transductant. The use of *att*λ deletions greatly facilitates finding the necessary Lac$^+$, prophage-containing, addition transductants since any phage containing transductants must necessarily have the prophage located in the *lac* region. It should be noted that when the λ*plac5* phage was integrated at *att*λ, we did not find phage containing the desired *lacZ* allele after the examination of over 25,000 plaques from an appropriate lysate.

The construction of genetically disabled λ*plac* transducing phages has facilitated analysis of those factors important in determining transduction frequency. λ*c*I857 *Nam7Nam53* phage establish lysogeny as plasmids in nonsuppressing strains (6, 18) and result in little cell death. The

TABLE 4. *Transduction of lacZ deletion strains*[a]

Recipient strain	*lacZ* deletion	Lac$^+$ transductants per ml
RDP 105	H111	5.8×10^5
RDP 106	H119	4.0×10^5
RDP 107	H114	3.3×10^5
RDP 108	H145	3.0×10^5
RDP 109	H125	1.2×10^5
RDP 110	H138	1.0×10^5
RDP 111	H220	5.1×10^4

[a] The indicated strains were transduced with λ*c*I857 *Nam7Nam53Pam80 placZ*$^+$ prepared from KL759 at an MOI of 2 as described in the text. The lactose selection plates were incubated at 37°C. The deletions have been described (11) and involve the deletion of increasing amounts of the *lacZ* gene carried by F128 starting from H111 up to H220, which has the least amount of *lacZ* DNA remaining.

additional inclusion of the λ *Pam*80 allele prevents the establishment of the plasmid state and provides a nonreplicating DNA molecule which cannot contribute to Lac^+ colony formation unless the transducing phage DNA recombines with the cellular DNA before it is lost by segregation (R. D. Porter, unpublished data). The data in Table 3 confirm our previous result that λ*plac* transducing phages recombine more readily with F42*lac* than with a chromosomal *lac* gene (13), and these new data show that this is the case regardless of the configuration of the *att*λ region of the chromosome. The transductants seen with λ*placZ*$^+$ in a cell that is *recA* when the cell contains a normal *att*λ site or a λ*ind* prophage would appear to be the result of site-specific recombination at *att*λ or at the hybrid attachment sites of the prophage, since strains that are both *recA* and Δ(*gal-att*λ-*bio*) (KL764 and KL770) fail to yield any transductants with λ*placZ*$^+$. This class of transductants probably arises because the transducing phage can lysogenize at *att*λ or at the hybrid attachment sites at a low efficiency as a result of λ*int* gene expression from the p_I *int* promoter (16). It would be theoretically possible to obtain Lac^+ transductants by site-specific recombination at secondary λ attachment sites even when *att*λ is deleted (17), but such integration of the transducing phage at secondary attachment sites apparently does not occur at significant levels in the present experiments. Similar results demonstrating a RecA dependence for plus-times-minus allele recombination have also been reported for λ*pro* and λ*gal* (19, 20). One of these reports indicated low but significant levels of transduction mediated by λ*red* functions in a *recA* strain with λ*gal* (19). We conclude from this that λ*red* functions are not significantly expressed in our disabled transducing phages.

With one notable exception (12), minus-times-minus transductional crosses have not been utilized for recombination studies in previous reports. The data in Table 3 indicate that *lacZ* × *lacZ* transductional crosses are totally dependent on host RecA gene function even when site-specific recombination at *att*λ is permitted. The data in Table 3 also point up the unexpected result that *lacZ* × *lacZ* crosses yield more Lac^+ recombinants than *lacZ*$^+$ × *lacZ* crosses when the recipient *lacZ* allele is located on F42*lac*, but not when the recipient *lacZ* allele is located on the chromosome. We have no explanation for this observation, but this phenomenon may point up some aspect of a possible mechanistic difference in the recombination between λ*plac* and F42*lac* versus F^-*lac*.

We have also used λ*plac* transduction as a measure of the ability of recombination to take place fairly randomly along the length of the recombining *lacZ* genes, or at least in rough proportion to the length of homology present. A disabled λ*placZ*$^+$ transducing phage lysate prepared from KL759 was used to transduce a series of strains containing deletions of various lengths in the resident *lacZ* gene. The data in Table 4 indicate that the level of transductants obtained is roughly proportional to the amount of the *lacZ* gene remaining in the recipient strain. Hence, the recombination seen between λ*plac5* derivatives and *lacZ* genes in recipient strains appears not to involve specific sites in the *lacZ* gene.

The λ*plac* transduction system has several advantages over previously utilized specialized transduction systems. Numerous well-characterized *lacZ* alleles are available and can be placed on the transducing phages. The separation of the *lac* region from *att*λ on the cellular genome allows the use of strains deleted for *att*λ, which is more difficult with λ*gal* transduction. The use of *att*λ deletions facilitates the analysis of general versus site-specific recombination in *lacZ*$^+$ × *lacZ* transductions. The use of the genetically disabled plaque-forming transducing phages described herein also allows transduction experiments to be done without the killing of cells in the recipient culture and without the presence of helper phage. This system should prove useful for further studies of recombination mechanisms.

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LITERATURE CITED

1. **Birge, E. A., and K. B. Low.** 1974. Detection of transcribable recombination products following conjugation in Rec^+, $RecB^-$ and $RecC^-$ strains of *Escherichia coli* K12. J. Mol. Biol. **83**:447–457.
2. **Campbell, A.** 1964. Transduction, p. 49–85. *In* I. C. Gunsalas and R. T. Stanier (ed.), The bacteria, vol. 5. Academic Press, New York.
3. **Echols, H., R. Gingery, and L. Moore.** 1968. Integrative recombination functions of bacteriophage λ; evidence for a site-specific recombination enzyme. J. Mol. Biol. **34**:251–260.
4. **Gottesman, M. E., and M. B. Yarmolinsky.** 1968. Integration negative mutants of bacteriophage lambda. J. Mol. Biol. **31**:487–505.
5. **Jordan, E., H. Saedler, and P. Starlinger.** 1968. 0° and strong-polar mutations in the *gal* operon are insertions. Mol. Gen. Genet. **102**:353–363.
6. **Lieb, M.** 1970. λ mutants which persist as plasmids. J. Virol. **6**:218–225.
7. **Low, B.** 1968. Formation of merodiploids in matings with a class of Rec^- recipient strains of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **60**:160–167.

8. **Low, B.** 1973. Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. J. Bacteriol. **113**:798–812.
9. **Low, K. B., and R. D. Porter.** 1978. Modes of gene transfer and recombination in bacteria. Annu. Rev. Genet. **12**:249–287.
10. **Malamy, M., H. Fiandt, and W. Szybalski.** 1972. Electron microscopy of polar insertions in the *lac* operon of *Escherichia coli*. Mol. Gen. Genet. **119**:207–222.
11. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
12. **Morse, M. L., E. M. Lederberg, and J. Lederberg.** 1956. Transductional heterogenotes in *Escherichia coli*. Genetics **41**:758–779.
13. **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.
14. **Shapiro, J. A.** 1969. Mutations caused by the insertion of genetic material into the galactose operon of *Escherichia coli*. J. Mol. Biol. **40**:93–105.
15. **Shapiro, J., L. MacHattie, L. Eron, G. Ihler, K. Ippen, and J. Beckwith.** 1969. Isolation of pure *lac* operon DNA. Nature (London) **224**:768–774.
16. **Shimada, K., and A. Campbell.** 1974. Int-constitutive mutants of bacteriophage lambda. Proc. Natl. Acad. Sci. U.S.A. **71**:237–241.
17. **Shimada, K., R. A. Weisberg, and M. E. Gottesman.** 1972. Prophage lambda at unusual chromosomal locations. I. Location of the secondary attachment sites and the properties of the lysogens. J. Mol. Biol. **63**:483–503.
18. **Signer, E. R.** 1969. Plasmid formation: a new mode of lysogeny by phage λ. Nature (London) **223**:158–160.
19. **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 Press, New York.
20. **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 Press, New York.
21. **Zipser, D.** 1967. Orientation of nonsense codons on the genetic map of the Lac operon. Science **157**:1176–1177.