ISOLATION OF P22 SPECIALIZED TRANSDUCING PHAGE FOLLOWING F′-EPISOME FUSION

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ABSTRACT

A P22 specialized transducing phage has been constructed which carries the structural gene for aspartate transcarbamylase (ATCase). This gene (*pyrB*) was first brought close to the P22 attachment site by fusing an F′ *pyrB*+ episome to an F′*prolac* episome which carries a P22 prophage attachment site. A prophage was added to these fused F′ episomes and the lysogen was UV-induced. The specialized transducing phage was isolated from the resulting lysate. The phage also carries *argI*, the structure gene for ornithine transcarbamylase.

SPECIALIZED transduction (as performed by phages λ and φ80) is restricted to bacterial genes adjacent to the prophage attachment site. Several methods have been used to circumvent this limitation and to construct specialized transducing phages carrying a variety of bacterial genes not normally located near the prophage attachment site. Three general methods have been used: (1) translocation of genes of interest to positions near the prophage (CUZIN and JACOB 1964; BECKWITH, SIGNER and EPSTEIN 1966; GOTTESMAN and BECKWITH 1969), (2) insertion of the prophage at an abnormal site near the genes of interest (SHIMADA, WEISBERG and GOTTESMAN 1972) and (3) fusion of two F′ episomes, one carrying the attachment site and the other carrying the bacterial genes of interest. This third method was first used by PRESS *et al.* (1971), who fused an F′ *trp att*$^{\phi 80}$ episome carrying the attachment site for the φ80 prophage with another F′ episome which includes methionine and arginine biosynthetic genes. This permitted formation of φ80 *arg* and φ80 *met* specialized transducing phages. The structure of such fused F′ episomes has been studied by WILLETS and BASTARRACHEA (1972) and by PALCHAUDHURI *et al.* (1972).

In the preceding paper (HOPPE and ROTH 1974) we have shown that Salmonella phage P22 recognizes an attachment site in an *E. coli* episome, F′ *pro*+ *lac*+. This makes it possible, by episome fusion, to bring a variety of genes close to the P22 prophage. We've used this technique to construct a P22 specialized transducing phage carrying the *E. coli pyrB* and *argI* loci. These genes code, respectively, for aspartate transcarbamylase and ornithine transcarbamylase. The genes are normally located far from the primary P22 prophage attachment site.

TABLE 1

	List of strains
TR132	*purC7 proA46 ile-405 gal-501* azaserine^{R}str^R/F′ *pro*+ *lac*+
TR1828	*pyrB648 argI537*
TR1857	*pyrB64 proAB47*
TR1969	*pyrB64 mel hisD6414*/F′$_{17}$ *pyrB*+
TR2081	*pyrB652 argI537*
TR2101	*pyrB64 mel*− *hisC527*(amber) *sup-501*(amber)
TR2256	*pyrB64* (P22) (P22 *pyrB*$_I$)
TR2257	*pyrB64* (P22) (P22 *pyrB*$_{II}$)
TR2274	*pyrB64 proAB47 cys-1508*/F′$_{17}$ *pyrB*+
TR2275	*purC7 proAB47 cys-1508*/F′$_{17}$ *pyrB*+-F′ *prolac*(fused)
TR2277	*recA-1 pyrB64* str^R
TR2279	*recA-1 pyrB64 proAB47* str^R
TR2280	*pyrB64 proAB47*(*ataA*−)/F′ *ataA*+ *pro*+ *lac*+
TR2476	*pyrB652 argI537*(P22) (P22 *pyrB*$_{II}$)

MATERIALS AND METHODS

Bacterial strains: Multiply-marked strains used are listed in Table 1. Mutant *proAB47* and strains carrying the *proAB47* deletion are derived from *S. typhimurium* strain LT7. All other strains are derived from LT2. The KLF′$_{17}$ *pyrB*+ episome was derived from the *E. coli* K12 HfrH by BROOKS LOW and was obtained from him; this episome is now designated F′$_{117}$ (LOW 1972). The F′ *prolac* episome is derived from *E. coli* K12 Hfr P804; this episome is now designated F′$_{128}$ (LOW 1972; E. SIGNER, personal communication). A Salmonella strain carrying the F′ *prolac* episome was obtained from JOE GOTS, University of Pennsylvania Medical School. The *rec*− mutation used in various strains is that described by WING, LEVINE and SMITH (1968); an Hfr strain (TR2246) carrying this *rec*− mutation was obtained from D. BOTSTEIN and used to construct the *rec*− strains in Table 1.

In order to select for fusion of F′$_{17}$ and F′ pro+ lac+, recipient strain TR2274 (*proAB47 pyrB64 cys-1508*/F′$_{17}$ *pyr*+) was mated with donor TR132 (*purC7 proA48 ile-405*/F′ *pro*+ *lac*+). Selection was made for simultaneous maintenance of both the F′ *pro*+ *lac*+ and F′$_{17}$ *pyr*+ episomes by requiring progeny to be PYR+ PRO+; the TR132 donor was counter-selected by requiring recombinants to be PUR+ and ILE+. Demands of this selection were met when the F′ *pro*+ *lac*+ episome was transferred into strain TR2274 and maintained by the progeny clone.

Mating was performed in the following way. A culture of TR2274 was grown 48 hours in nutrient broth with aeration by shaking. The prolonged incubation in stationary phase was done to generate F− phenocopies. A logarithmically growing culture (1×10^8 cells/ml) of TR132 was used as donor. Two-ml aliquots of each of these two cultures were mixed and the cells collected on a millipore filter. The filter membrane carrying the mixture of cells was placed on a prewarmed (37°) nutrient broth plate for 40 minutes to permit contact formation and mating initiation. The filter was then transferred to a flask containing 50 cc of liquid nutrient broth and was shaken gently for 1 hour to permit maximum transfer. Cells from this culture were collected on a millipore filter and washed with E medium containing 0.2*mM* cysteine. The filter was placed in 25 cc of E medium containing 0.2*mM* cysteine and the cells were grown overnight to select the desired PYR+ PRO+ recombinant. The resulting culture was diluted 1:50 into the same selective medium and grown to saturation. Finally this culture was diluted 10^6-fold and plated for single colonies on plates containing E medium plus cysteine. These colonies were then checked for possession of fused F′ elements.

Other fusion episomes were generated by plating the two F′-carrying strains directly on selective medium; this method has the advantage that each colony arising must be the result of an independent event.

Lysogenization of fused F′ episomes: Phage P22 was plated for single plaques upon strain TR2275, which carries one of the fused F′ episomes which were generated. Cells lysogenic for P22 were picked from the turbid centers of the phage plaques and single-colony-isolated on nutrient broth. The chromosomal prophage attachment site (*ataA*) of strain TR2275 is deleted by the *proAB47* mutation; therefore the only *ataA* site available is located on the fused F′-episome. The lysogens obtained proved to have the prophage associated with the fused F′ episomes. Lac⁻ segregants uniformly lose the prophage with the rest of the F′-episomal markers. Thus the *ataA* region of the F′ *pro*+ *lac*+ was maintained when the F′ fusion occurred.

Replica plate method for scoring high frequency transduction: When a low-frequency transducing lysate was used to obtain PYR+ transductants, it was necessary to determine which of these transductants were due to rare generalized transduction and which were due to the desired specialized transducing phage. To determine this, transductants were UV-induced and the resulting lysates tested for their ability to transduce PYR+. Transductants carrying a P22 *pyrB* specialized transducing phage would be expected to give rise to lysates which show a high frequency of PYR+ transduction.

In order to screen a large number of transductants, these tests were performed on solid medium. Transductant clones (PYR+) were transferred to a plate of minimal agar medium (24 clones/plate) and grown overnight. These plates were replica-printed onto nutrient agar and allowed to grow 4–6 hours at 37°. At this time all the patches of cells showed detectable growth. The plates were then UV-irradiated (20 seconds at 40 ergs/mm²/second) to induce phage production. Following irradiation, the plates were incubated 5 hours at 30° to permit phage production. Cells were then killed by inverting each plate over chloroform at room temperature for 10 minutes. This leaves each plate with 24 areas of phage and no viable cells. These plates were then replica-printed onto lawns of a recipient strain (*pyrB64*) on selective medium (E medium plus 0.4 ml nutrient broth/plate). The low level of supplementation permitted some growth of the recipients and enhanced transduction frequencies. In this manner, 24 transduction crosses could be performed on a single plate. Scoring the number of transductant clones per phage patch indicated whether or not the original transductant clone carried a specialized transducing phage.

RESULTS

1) E. coli *and* S. typhimurium *recombine poorly in the* pyrB *region.*

In searching for a specialized transducing phage for the *pyrB* region we wish to establish conditions such that the *pyrB* region is transducible *only* or predominantly by specialized transduction. Thus conditions must be found to minimize generalized transduction of the *pyrB* region. Transduction of an *E. coli pyrB* region into Salmonella seems to provide such conditions. In general, true recombination between the chromosomes of *E. coli* and *S. typhimurium* is very infrequent, presumably due to poor base sequence homology. This lack of homology would be expected to greatly reduce generalized transduction while leaving specialized transduction unimpaired, since the latter depends only on the *site-specific* recombination system used for lysogenization by the specialized transducing phage.

The *pyrB* regions of Salmonella and *E. coli* recombine very poorly with each other. This was shown by generalized transductional crosses. A donor Salmonella strain (TR1969) was constructed which carries the *pyrB64* mutation and an *E. coli* F'_{17} *pyrB*+ episome. *The only functional pyrB region of this strain is derived from E. coli.* P22-mediated transductional crosses were performed using a Salmonella *pyrB64* mutant as recipient. Results are presented in Table 2. When PYR+ recombinants are selected, recombinational events are required between

TABLE 2

Recombination between the pyrB *regions of* E. coli *and* S. typhimurium

Recipient	Recombinant type selected	Donor:	Transductants/1.4×10^{10} p.f.u TR1969 (*pyrB64*/F′$_{17}$ *pyrB*+)	LT2
pyrB64	PYR+		2	2418
proB25	PRO+		2277	2314

These generalized transductional crosses were performed using P22(*int-4*) phage grown lytically on the indicated donor strains. Donor strain TR1969 is a Salmonella strain harboring the *E. coli* episome, F′ *pyrB*+; the only functional *pyrB* region in this strain is of *E. coli* origin. Strain LT2 is a wild-type *S.typhimurium* strain. All of the crosses were performed at multiplicities less than 25 phage particles per recipient cell. In each case, a total of 1.4×10^{10} donor phage (p.f.u.) were tested on several transduction plates.

the *E. coli pyrB*+ region of the donor and the *S. typhimurium pyrB*− region of the recipient. In contrast, when PRO+ recombinants are selected using mutant *pro25* as recipient, the recombinational events involve only *Salmonella* material. Very few PYR+ recombinants arose, suggesting that little recombination occurs between *E. coli* and *S. typhimurium* chromosomes in this region; PRO+ recombinants arose with high frequency. In Salmonella-Salmonella crosses (using donor phage grown on wild-type strain LT2) PRO+ and PYR+ recombinants appear at approximately the same frequency. Thus use of the *E. coli* material reduces generalized transduction in this chromosomal region by about 1,000-fold.

2) *Episome fusion.*

Only genes located near the prophage site are subject to inclusion in specialized transducing phages. The *pyrB* gene and the P22 prophage attachment site are normally separated by almost a quarter of the chromosome. In order to bring the *pyrB* region near the P22 prophage, we selected for fusion of the *E. coli* episomes F′$_{17}$ and F′ *pro*+ *lac*+. The *pyrB*+ gene is present on the F′$_{17}$ episome, and the P22 attachment site (*ataA*) is present on the F′ *pro*+ *lac*+ episome. When selection is made for simultaneous maintenance of both episomes, fusion of the two F′ episomes is selected; this fusion is required in order to overcome the normal tendency of separate F′ episomes to exclude each other (Scaife and Gross 1962; DeHaan and Stouthamer 1963; Echols 1963). Such fusion brings the *pyrB* and *ataA* loci closer together. Among a number of such fused episomes we hoped to find one in which the two loci were sufficiently close to permit formation of a P22 *pyrB* specialized transducing phage.

The steps involved in episome fusion are diagrammed in Figure 1. First the F′ *pro*+ *lac*+ episome is transferred into TR2274 selecting PYR+ PRO+ [ILE+ PUR+]. The procedures used for selection of strains carrying fused episomes are described in materials and methods. All clones resulting from fusion events were found to be LAC+ and carry a fused episome containing material derived from both F′$_{17}$ and F′ *pro*+ *lac*+.

Two lines of evidence demonstrate that episome fusion has occurred. First, LAC− segregant clones, which are easily isolated, are all found to be PRO− and

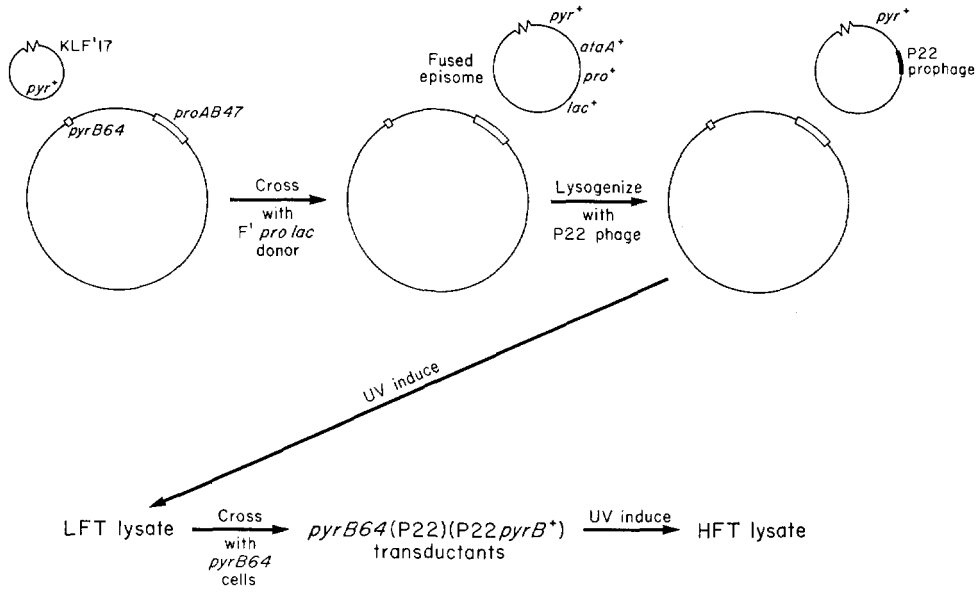

FIGURE 1.—Sequence of events involved in construction of a high-frequency *pyrB* transducing lysate. Details of each step are described in the text.

PYR⁻. Thus the *lac*⁺, *pro*⁺ and *pyr*⁺ genes are lost together. This is consistent with these genes being located on a single plasmid, which is occasionally lost. The second line of evidence is co-transfer of the *lac*⁺, *pro*⁺ and *pyr*⁺ genes. When strains carrying the putative fused episomes are used as donors and selection is made for transfer of *lac*⁺, the progeny always receive the *pro*⁺ and *pyr*⁺ genes as well. Conversely, selection for transfer of the *pyr*⁺ gene yields progeny which have also received the unselected *lac*⁺ and *pro*⁺ genes. Four, independently generated, fusion-F′ episomes were constructed (see MATERIALS AND METHODS) and used in the search for specialized transducing phage. Each of the four strains was lysogenized with wild-type P22. Thus four different strains were constructed; in each, the P22 prophage is associated with a fused episome carrying the *pyrB* gene.

3) *Search for specialized transducing phage.*

The four strains carrying fused episomes with P22 prophages were UV-induced to prepare low frequency transducing lysates. The lysates were used in transduction crosses with recipient strain *pyrB64*, and selection was made for PYR⁺ transductants. Any PYR⁺ transductants arising from specialized transduction should retain the original *pyrB* mutation and incorporate by lysogeny an additional *pyrB* region carried by a P22 *pyrB*⁺ transducing phage generated during UV-induction of the donor. Presumably some of these transductants could also be rare "true" recombinants between the *E. coli pyrB* region of the donor and the *S. typhimurium pyrB* region of the recipient. In order to identify transductants containing a specialized transducing phage, several hundred transductants from the four donor strains were tested (as outlined in MATERIALS AND METHODS) for

TABLE 3

*Dependence of P22*pyrB *phages on* recA *and* ataA

Recipient strain			Donor phage (p.f.u.) required for one PYR+ transductant		
			Specialized transduction		Generalized transduction
Relevant genotype		Strain designation	P22*pyrB*$_{I}$	P22*pyrB*$_{II}$	P22 grown in LT2
recA+	*ataA*+	*pyrB64*	1.6×10^{6}	44	4.7×10^{6}
recA+	*ataA*−	TR1857	1.6×10^{6}	912	3.7×10^{6}
recA−	*ataA*+	TR2277	3.3×10^{9}	650	$> 2 \times 10^{10}$
recA−	*ataA*−	TR2279	1.9×10^{10}	5.3×10^{5}	$> 2 \times 10^{10}$

Specialized transduction was performed using lysates prepared by UV induction of TR2256 [*pyrB64*(P22)(P22 *pyrB*$_{I}$)] and TR2257 [*pyrB64*(P22)(P22 *pyrB*$_{II}$)]. UV induction and phage preparation was done as described by HOPPE and ROTH (1974).

Generalized transduction was performed using P22 mutant *int-4* grown lytically on *S. typhimurium* wild type, LT2.

release of specialized transducing phage. Among the transductants obtained from one of the four lysates were five which seemed to release transducing phage.

One transductant (TR2256, derived from fused episome strain TR2255) was chosen for further study. After UV-irradiation, a lysate of TR2256 was prepared which transduced the *pyrB* region rather poorly (See P22 *pyrB*$_{I}$ in Table 3). Although the transducing efficiency of this lysate is low (1 transductant/10^{6} p.f.u.) we were encouraged because this is so much more efficient than generalized transduction of the same *E. coli pyrB* region into Salmonella (1 transductant/10^{10} p.f.u.; Table 2). The new lysate transduces the *E. coli* material over 1,000-fold more efficiently than does generalized transduction of the same material. Therefore we considered it likely that the lysate contains a specialized transducing phage (P22 *pyrB*$_{I}$). As seen in Table 3, P22 *pyrB*$_{I}$ also transduces (although poorly) a *rec*$^{-}$ recipient to PYR^{+}; generalized transduction does not occur in this *rec*$^{-}$ strain.

4) *Isolation of an improved specialized transducing phage* (*P22* pyrB$_{II}$).

Since transduction by the P22 *pyrB*$_{I}$ lysate is so inefficient, any variants of P22 *pyrB*$_{I}$ with high transducing efficiency should contribute heavily to the transduction efficiency of the lysate. Such variants should be readily detected by screening transductant clones. In search of such an improved variant, we used P22 *pyrB*$_{I}$ lysates to transduce *pyrB64* cells to prototrophy. A large number of these transductants were screened to see if any could be found whose lysates had higher frequency transducing ability.

Among several hundred transductants, one (TR2257) was chosen which gave lysates with a transducing ability substantially higher than the original P22 *pyrB*$_{I}$ lysates; transduction efficiency is approximately one transductant per 50 p.f.u. The improved transducing phage is designated P22 *pyrB*$_{II}$. All further characterization involves this improved phage.

5) *Dependence of transduction by P22* pyrB$_{II}$ *on* rec *and* ataA.

In Table 3 it can be seen that P22 *pyrB*$_{II}$ lysates give about 1 transductant per 50 plaque-forming units. If the recipient strain lacks the primary P22 attach-

ment site (*ataA*) transduction efficiency is reduced over 10-fold. The *ata* deletion has no effect on generalized transduction. Recipients having a *recA* mutation are not transducible by generalized transduction, but can be transduced by P22 $pyrB_{II}$ with only a 10-fold reduction in efficiency (Table 3, line 3). The reduction caused by the *rec* mutation may reflect the contribution of *rec* to phage circularization or it may be due to poor viability of rec^- transductants.

6) *Phage P22* $pyrB_{II}$ *also carries the* argI *gene.*

The *argI* gene codes for ornithine transcarbamylase. In both *E. coli* and *S. typhimurium* the *argI* gene is very close to the *pyrB* gene (GLANSDORFF, SAND and VERHOEF 1967; SYVANEN and ROTH 1972). Thus it seemed possible that P22 $pyrB_{II}$ might include both the *argI* and *pyrB* genes. This was tested using *argI pyrB* double mutant (TR1828) as a recipient in transduction crosses. Selection was made for PYR^+ or for ARG^+ transductants, and recombinants were scored for inheritance of the wild-type donor allele of the other locus. These results are presented in Table 4. Using generalized transduction, *pyrB* and *argI* show 62% or 49% cotransduction, depending on which is the selected marker. When P22 $pyrB_{II}$ is used in transduction, these two genes are always transduced together. This is expected if both genes are present in a viral genome that is added to the chromosome as a unit by lysogeny. It is likely that P22 $pyrB_{II}$ phage also carries the gene for the regulatory subunit of aspartate transcarbamylase, since this gene seems to be adjacent to the *pyrB* locus (SYVANEN and ROTH 1973).

7) *P22* $pyrB_{II}$ *transductants are merodiploid.*

Transductants obtained by crossing P22 $pyrB_{II}$ lysates with *pyrB64* are expected to have the genotype: *pyrB64* (P22) (P22 $pyrB_{II}$). That is, they are expected to retain the chromosomal *pyrB* mutation and to carry the transduced, phage-associated, $pyrB^+$ gene at a different site (presumably the prophage attachment site). As expected, the *pyrB64* mutation is still present in these PYR^+

TABLE 4

Co-inheritance of argI *and* pyrB *in generalized and specialized transductional crosses*

Recipient	Donor phage	Transduction type	Selected recombinant	Percent carrying unselected donor marker
pyrB648 argI537 (TR1828)	P22	Generalized	Pyr+	62% Arg+ (186/300)
			Arg+	49% Pyr+ (147/300)
pyrB648 argI537 (TR1828)	P22 $pyrB_{II}$	Specialized	Pyr+	100% Arg+ (300/300)
			Arg+	99% Pyr+* (297/300)

* The few Arg^+ Pyr^- clones are probably Arg^+ revertants of the recipient strain since the *argI537* mutation has an appreciable revertant frequency.

P22 used as generalized transducing phage is the non-lysogenizing mutant, *int-4*, grown lytically on LT2. The P22 $pyrB_{II}$ specialized transducing lysate was made by UV induction of TR2257. Results essentially identical to those above were obtained using a different recipient strain, TR2081 (*pyrB652, argI537*).

transductants. This was shown by recovering a *pyrB64* mutation from several PYR$^+$ transductant clones.

Generalized transducing phage KB was used because it is not coimmune with P22 and will grow on the P22-lysogenic transductants. Phage KB was grown on three different PYR$^+$ transductants whose genotype is believed to be: *pyrB64* (P22) (P22 *pyrB*$^+_{II}$). The KB lysate was used to transduce the *argI537* mutant to ARG$^+$; and recombinants were scored for inheritance of the closely-linked *pyrB64* mutation. All three lysates gave ARG$^+$ PYR$^-$ transductants, showing that the PYR$^+$ donor strain does contain a *pyrB* mutation. This is strong evidence that PYR$^+$ transductants obtained using P22 *pyrB*$_{II}$ are merodiploid.

If the transducing phage were lost from a merodiploid transductant, a PYR$^-$ clone should result. Such transducing phage loss is common for the P22 specialized transducing phages described earlier (HOPPE and ROTH 1974). The P22 *pyrB*$_{II}$ prophage, however, seems to be extremely stable. Even after penicillin selection, no PYR$^-$ clones were detected in cultures of TR2257 [*pyrB64* (P22) (P22 *pyrB*$_{II}$)]. In addition to TR2257, several other, independently selected, P22 *pyrB*$^+_{II}$ transductant clones were also tested; these were obtained by exposing TR1828 (*argI*$^-$ *pyrB*$^-$) to P22 *pyrB*$_{II}$ phage prepared by induction of TR2257. Two transductants, chosen at random, were PYR$^+$ ARG$^+$ (see Section 6) and gave rise to PYR$^-$ ARG$^-$ segregants at a very low frequency (0.1% segregants following penicillin selection for auxotrophs). Another transductant of TR1828 was chosen because it seemed to release P22-sensitive segregants (identifiable on indicator agar). This clone gave PYR$^-$ ARG$^-$ segregants at a very high frequency: presumably this unstable clone carries a varient of the P22 *pyrB*$_{II}$ prophage. In these cases, it was shown that segregants occur which lose both *pyrB*$^+$ and *argI*$^+$ genes together. This is strong evidence that P22 *pyrB*$^+_{II}$ prophage (which contains both *pyrB* and *argI* genes) can be lost as a unit, although this loss is normally a very infrequent event. Reasons for this stability will be discussed below (see DISCUSSION).

8) *Phage P22* pyrB$^+_{II}$ *integrates at the prophage attachment site.*

It would be expected that specialized phage P22 *pyrB*$^+$ would transduce by inserting into the prophage attachment site, *ataA*, of the recipient. Use of the *ataA* insertion site has already been suggested since recipients lacking the *ataA* site show a 10-fold reduction in transduction efficiency (Table 3). Insertion at the *ataA* site was tested directly by use of a strain (TR2280) whose only *ataA* locus is on the F′ *pro*$^+$ *lac*$^+$ episome. Strain TR2280 [*pyrB64 proAB47* (*ataA*$^-$)/ F′ *ataA*$^+$ *pro*$^+$ *lac*$^+$] carries both a *pyrB* mutation and a deletion (*proAB47*) which removes the chromosomal prophage attachment site (*ataA*).

This strain was infected with P22 *pyrB*$_{II}$ phage and PYR$^+$ transductants were selected. Six of these PYR$^+$ transductants were purified and then allowed to lose their F′ *pro*$^+$ *lac*$^+$ episomes. Segregant clones which had lost their episomes were picked as LAC$^-$ colonies on EMB lactose indicator plates. The LAC$^-$ segregants derived from all six transductants had become simultaneously LAC$^-$, PRO$^-$, PYR$^-$ and P22-sensitive. Thus the *pyrB* genes and the helper phage, which is pre-

sumed to accompany it, associate with the F′ *pro lac* episome. This is strong evidence that P22 $pyrB_{II}$ inserts into the *ataA* locus and not into the *pyrB* region of the chromosome.

9) *Phage P22* $pyrB_{II}$ *does not require helper phage for transduction.*

The P22 $pyrB_{II}$ lysates that have been used thus far are presumed to contain both P22 $pyrB_{II}$ phage and also wild-type P22 helper phage. The following experiments support this presumption and indicate that the helper is not required for transduction.

When P22 $pyrB_{II}$ lysates are used at very low multiplicity in transduction crosses, there is no drop in the number of transductants obtained per plaque-forming unit (see Table 5). Under these conditions it is impossible for a recipient cell to be infected by both a transducing phage particle and a helper phage particle. Therefore we feel that the helper phage is not required for transduction. In Table 5 it can be seen that addition of excess helper does not enhance transduction efficiency more than a fewfold; this supports the conclusion that helper phage is not required for transduction. The observed slight increase in Pyr^+ transductants with helper may be due to an increase in the probability of lysogeny at high multiplicity of infection.

If no helper is required, then the transductants obtained using very low donor phage multiplicity of infection should contain only the P22 $pyrB_{II}$ prophage and no wild-type helper prophage. Twenty-one PYR^+ transductants were tested which had arisen following crosses with fewer than 30 plaque-forming units per plate (Table 5, lines 3 and 4). All of these transductants were found sensitive to P22 infection and all failed to release viable P22 phage. Thus we conclude that these transductants contain only a defective P22 $pyrB_{II}$ prophage, and no wild-type P22 prophage is present.

TABLE 5

*Effect of helper phage on transduction by P22*$pyrB_{II}$

P22 $pyrB_{II}$ donor phage (p.f.u.) per plate	PYR^+ transductants found per 100 donor phage (p.f.u.)	
	— Helper	+ Helper
2.7×10^3	3.9 (211)*	9.4 (527)*
2.7×10^2	3.3 (46)	11.5 (154)
27	5.6 (15)	10.4 (28)
2.7	22.2† (6)	11.1† (3)

Each plate was seeded with 2×10^8 cells of recipient strain TR2101 (*pyrB64 sup-501*) and the indicated number of donor phage plaque-forming units. Donor phage P22 $pyrB_{II}$ was prepared by UV irradiation of strain TR2257 [*pyrB64*(P22)(P22 $pyrB_{II}$)]. Helper phage (P22 amber mutant *amN7*) was used at a multiplicity of 10 p.f.u. per recipient cell. Helper phage was prepared by lytic growth on TR2101 and shows no ability to transduce the recipient to Pyr^+.

* The numbers in parentheses indicate the number of transductants counted to determine the transduction efficiency.

† In these cases, determination of efficiency is based on so few transductants that the exact value is uncertain. It seems clear, however, that low multiplicity of infection by the donor phage does not impair transduction efficiency.

10) *Phage genes associated with P22* pyrB$_{II}$.

Most PYR$^+$ transductants obtained carry both P22 *pyrB*$_{II}$ and wild-type P22 helper phage. Two approaches were taken to determine what phage genes are retained by the P22 *pyrB*$^+_{II}$ phage itself, independent of helpers. First, transductants were sought which had become PYR$^+$ but not phage-resistant. These, we hoped, would represent transductants which had received the transducing phage but not the wild-type P22 helper. These transductants were obtained by performing transduction at very low phage multiplicities (see preceding section) or by using TR1857 [*pyrB64 proAB47* (*ataA*$^-$)] as a recipient. [The absence of a prophage attachment site makes it possible to isolate phage-sensitive transductants even at high donor phage multiplicities (HOPPE and ROTH 1974)]. Various P22 amber mutants were plated on these phage-sensitive transductants to see what phage genes could be supplied by cells carrying the P22 *pyrB*$_{II}$ prophage. The phage-sensitive transductants were unable to support the growth of any of the P22 amber mutants tested. (Mutants tested carried lesions in the following P22 genes: 1, 2, 3, 5, 8, 9, 10, 12, 13, 16, 19, 20, 22, 23, 26.) Thus, by this means, no evidence was found of phage genes associated with the *pyrB* genes of P22 *pyrB*$_{II}$.

Although no phage genes were detected in the preceding experiment, P22 *pyrB*$_{II}$ must carry some phage material since it inserts at the P22 prophage attachment site (see above). Even in the absence of a helper phage genome, the *pyrB* genes of P22 *pyrB*$_{II}$ insert at the prophage attachment site. This is shown by the following experiment.

In the course of a transductional cross using a lysate containing P22 *pyrB*$^+_{II}$ phage and recipient strain TR2280 [*pyrB64 proAB47* (*ataA*$^-$)/F′ *pro*$^+$ *lac*$^+$], several phage-sensitive PYR$^+$ transductants were obtained. Apparently the transducing phage had inserted and formed a prophage without a helper. These transductants, like those discussed above, showed no evidence of functional phage genes. If these transductants arise by specialized transduction, one would expect the *pyrB*$^+$ genes to be located at the prophage attachment site. Since the recipient strain's only prophage attachment site is on the F′ *pro*$^+$ *lac*$^+$ episome, the *pyrB*$^+$ genes should be associated with the F′ *pro*$^+$ *lac*$^+$. This was found to be true. When these transductants were allowed to lose their F′ *pro*$^+$ *lac*$^+$ episomes, they simultaneously became LAC$^-$, PRO$^-$, and PYR$^-$. This suggests that the P22 *pyrB*$_{II}$ genome was inserted at the P22 attachment site on the F′ episome and yet contained no phage genes detectable by our methods.

10) *Density of P22* pyrB$^+_{II}$ *phage.*

When P22 *pyrB*$_{II}$ phage is centrifuged in a cesium chloride density gradient, it bands at a position very close to that of P22. Thus the DNA content per phage particle seems generally similar to that of P22. This is expected for phages which package DNA by some sort of "measuring" mechanism. The very slight density difference between P22 and P22 *pyrB*$_{II}$ is probably due to differences in base composition of DNA.

DISCUSSION

The following lines of evidence lead us to conclude that P22 $pyrB_{II}$ is a specialized transducing phage.

a) The transducing efficiency is high (approximately one transductant per 50 plaque-forming units).

b) Transduction occurs in *recA*$^-$ recipient strains. This indicates that transduction does not depend on true recombination. Such behavior is expected if the transducing phage genome can be added to the chromosome by the phage site-specific recombination mechanism.

c) In transductants, the *pyrB* gene donated by P22 $pyrB_{II}$ seems to be located at the P22 prophage attachment site. This is supported by the twentyfold drop in transduction efficiency seen when the recipient strain lacks the primary P22 prophage attachment site, *ataA*. When the only *ataA* site in the recipient is on a F′ *prolac* episome, the *pyrB* gene becomes associated with the F′ element.

While the above properties are expected for a specialized transducing phage, several additional properties of P22 $pyrB_{II}$ are surprising.

d) Thus far no phage genes have been detected for this transducing phage.

e) The specialized transducing phage genome is quite stable; the transduced material is very rarely lost. In contrast, all the P22 specialized phages described in the preceding paper (P22 *pro*, P22 *prolac* and P22 *arg*) give unstable transductants.

This failure to detect phage genes is particularly disturbing. Since this phage transduces *rec*$^-$ strains well, the phage must provide some means of circularizing and inserting the incoming fragment. In a wild-type P22 infection the *erf* gene can provide for circularization of the genome; the *int* gene is required for prophage insertion. Permissive rescue experiments have failed to reveal an *erf* gene associated with the P22 $pyrB_{II}$ transducing phage. We have not yet checked P22 $pyrB_{II}$ for possession of an *int* gene. The circularization and insertion mechanism of P22 $pyrB_{II}$ remains to be clarified. It is probable that transducing phage P22 $pyrB_{II}$ will prove to possess at least a small bit of the P22 genome.

The stability of the material transduced by P22 $pyrB_{II}$ may be a consequence of the paucity of phage material. The instability seen for other transducing phage genomes may be a consequence of the tandem location of helper phage and transducing phage genomes in the chromosome. If the transducing phage carries material homologous to the helper, then the transducing phage might loop out and be excised by a recombinational event within the duplicated region. Since P22 $pyrB_{II}$ contains very little phage material, the duplicated region would be small and this may reduce the frequency of excision.

The position of the bacterial genes relative to the duplicated material might also affect the stability of transductants. If the transducing phage and helper insert so that the bacterial genes are not between the helper prophage and the phage genes of the transducing phage, then looping out will cause helper loss rather

than loss of the transducing phage genome. This possibility is supported by the observation that transductants carrying both P22 $pyrB_{II}$ and helper P22 lose the helper phage genome with relatively high frequency.

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