SPECIALIZED TRANSDUCING PHAGES DERIVED FROM PHAGE P22 THAT CARRY THE *proAB* REGION OF THE HOST, *SALMONELLA TYPHIMURIUM*: GENETIC EVIDENCE FOR THEIR STRUCTURE AND MODE OF TRANSDUCTION

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Manuscript received July 1, 1975
Revised copy received March 5, 1976

ABSTRACT

Two independently isolated specialized transducing phages, P22*pro-1* and P22*pro-3*, have been studied. Lysates of P22*pro-1* contain a majority of transducing phages which can go through the lytic cycle only in mixed infection; these defective phages transduce by lysogenization in mixed infection and by substitution in single infection. A few of the transducing phages in P22*pro-1* lysates appear to be non-defective, being able to form plaques and to transduce by lysogenization in single infection. Transduction by P22*pro-3* lysates is effected by non-defective transducing phages, which transduce by lysogenization; these lysates also contain a majority of defective phages which do not co-operate in mixed infection.

The P22*pro-1* genome is thought to contain an insertion of bacterial DNA longer than the terminal repetition present in P22 wild type, so that at maturation a population of differently defective phages is produced. The exact structure of the P22*pro-3* genome is open to conjecture, but it seems clear that the insertion of bacterial DNA is smaller than that in P22*pro-1*. Both P22*pro-1* and P22*pro-3* are defective in integration at *ataA* under non-selective conditions, although both integrate on medium that lacks proline.

TRANSDUCTION of certain genes of *Salmonella typhimurium* by phage P22 can be effected by specialized transducing particles. Genes for which specialized transduction has been reported are the *pro* genes, which are located near the prophage attachment site (SMITH-KEARY 1966; WING 1968; SMITH 1968; JESSOP 1972), and *tetR* genes, which are located on an *R* factor (WATANABE *et al.* 1972).

The properties of the specialized transducing phages would be expected to reflect the fact that P22 is a circularly permuted, terminally repetitious, phage (RHOADES, MACHATTIE and THOMAS 1968), encapsulating at maturation "headfuls" of DNA from oversize DNA molecules (STREISINGER, EDGAR and DENHARDT 1964; BOTSTEIN and LEVINE 1968). Circularization of the infecting phage DNA molecule by recombination within the terminal repetition seems to be essential to growth and lysogenization (BOTSTEIN and MATZ 1970). CHAN *et al.* (1972) found that specialized transducing phages carrying *tetR* genes were unable to lysogenize or go through the lytic cycle in single infection, but that different phage particles in a lysate could co-operate in mixed infection. They interpreted

TABLE 1

Strains

Bacterial strains	Relevant characters, and sources	
proA15 su⁻ ara+	Point mutant in *proA* gene; non-suppressing host for P22*am*; able to ferment arabinose. Since the *su⁻* and *ara+* characters were only used in a couple of experiments, the strain is usually referred to as *proA15*.	WING (1968)
proAB47	Deletion covering the whole of the *proAB* region, and *ataA* (the primary attachment site for P22).	MIYAKE and DEMEREC (1960)
su+ pro⁻	Suppressor of P22*am* mutants. Mutant in *proAB* region.	KOLSTAD and PRELL (1969)
DB136 DB147	Defective lysogens for P22; deletions extend into the prophage from *proA* (see Figure 1).	CHAN and BOTSTEIN (1972)
DB5057*pro⁻* DB5201*pro⁻*	Defective lysogens for P22; deletions extend into the prophage from the end opposite to *proA* (see Figure 1). Mutant in the *proAB* region.	CHAN and BOTSTEIN (1972)
proC437	Mutant in *proC* gene; isolated by the author.	
Phage strains		
P22 WT	Wild-type strain of P22.	
P22 c_1	Mutant in c_1 gene for establishment of lysogeny.	LEVINE (1957)
P22 c_2	Mutant in c_2 gene for establishment and maintenance of lysogeny.	LEVINE (1957)
P22 c_3	Mutant in c_3 gene for establishment of lysogeny.	LEVINE (1957)
P22L4*int⁻*	Mutant in gene for integrase.	SMITH and LEVINE (1967)
P22*amH79*	Amber mutant in late gene.	KOLSTAD and PRELL (1969)
P22*amH80*	Amber mutant in early gene.	KOLSTAD and PRELL (1969)
P22*pro-1*	P22 carrying *proAB* region.	JESSOP (1972)
P22*pro-3*	P22 carrying *proAB* region.	JESSOP (1972)
P22*n-1*	Non-transducing derivative of P22*pro-1*.	
P22*n-3*	Non-transducing derivative of P22*pro-3*.	

their data to mean that P22*tetR* phages carry bacterial DNA inserted into the phage genome, so that at maturation the DNA encapsulated lacks terminal redundancy and a few phage genes; since P22 is circularly permuted, the transducing phages would each be defective for a different part of the phage genome. Subsequently heteroduplex mapping has shown that the insertion of bacterial DNA in a P22*tetR* transducing phage is 20% of the phage genome (TYE, CHAN and BOTSTEIN 1974). P22 has a terminal repetition of 2%, so the finding of a 20% insertion confirmed the structure predicted from the genetic properties.

Properties of a specialized transducing phage carrying the *proAB* region have suggested that its lysates contain some transducing phages able to form plaques in single infection (JESSOP 1972). The results of further studies on this phage, P22*pro-1*, and on an independently isolated transducing phage, P22*pro-3*, are being presented in this paper.

MATERIALS AND METHODS

Strains

Bacterial and phage strains are listed in Table 1. Mutant *proAB47* is derived from *Salmonella typhimurium* LT7; all the other bacterial strains are derived from LT2. The *pro* mutations carried by *su*+-*pro*⁻, DB5057 and DB5201, were isolated by the author, by enriching for spontaneous mutations by penicillin screening; cross-feeding with a *proC* mutant showed that these mutations were in the *proAB* region. P22*pro-1* and P22*pro-3* were isolated from transductants derived from *proAB47* transduced by P22 wild type grown on LT2 wild type (JESSOP 1972). The phages were named P22-*p-pro-1* and P22-*p-pro-3* respectively, on account of the fact that *pro*+ cells could be recovered from their plaques; however, in view of the fact that lysates of both phages contain large numbers of defective, as well as of non-defective, transducing phages, the *p* is now omitted from the names. The phage preparations used in the experiments were obtained by induction of transductants of *proA15*; these transductants originated from transductions where the multiplicity of infection was approximately 10^{-6}. Plaques formed by both transducing phages are small and irregular in shape.

P22*n-1* and P22*n-3* are non-transducing phages derived from P22*pro-1* and P22*pro-3* respectively. Both these transducing phages may lose their *pro* genes, so that lysates invariably contain a proportion of non-transducing phages, readily distinguishable by the fact that they form large plaques. The phage preparations used in the experiments described in the paper were obtained by induction of transductants derived from *proAB47* infected at low multiplicities of infection; for reasons not yet understood some transductants of this kind yield lysates containing a majority of non-transducing "revertants".

Media

These were as previously described (JESSOP 1972), except that tryptone agar was used as base medium for phage assays (CLOWES and HAYES 1968). Eosin-methylene base agar (—lactose) supplemented with 2% arabinose was used in experiments on the frequency of lysogenization.

Methods

All experiments were carried out at 37°. Asssays for plaque-forming particles were done on log phase cultures, concentrated from 2×10^7/ml to 2×10^8/ml; the small P22*pro* plaques become quite large if the indicator is prepared thus. In the phage assays and in the transductions the exact multiplicities of infection were not determined; at most phage concentrations the ratio of phage to bacteria reflects the multiplicity of infection fairly accurately, so the term m.o.i. is often used, for the sake of brevity.

The frequency of the reductive response was tested for by the method of LEVINE (1957), using an *ara*+ strain as host, and an *ara*⁻ strain as indicator.

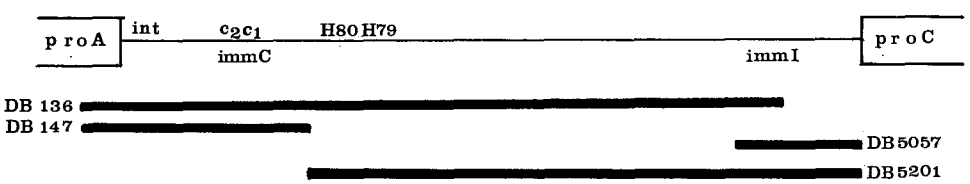

FIGURE 1.—Prophage map of P22 showing the four prophage deletions, and mutants used in the experiments.

The stability of lysogens was tested as follows. Lysogenic cells from the centers of plaques (formed by phages plated at high or at low multiplicities of infection) were streaked onto complete medium to give single colonies. Whether or not lysogenic cells were segregated was ascertained by testing these single colonies (1) for immunity to superinfecting phage, by cross-streaking against phage P22 c_2 (SUSSKIND and WRIGHT 1972) and (2) for the release of phage by replicating onto phage-sensitive indicator (TAYLOR 1963). Fifteen to twenty colonies were tested from each lysogen. Lysogens derived from the P22*pro* phages, and from their non-transducing derivatives, were compared with lysogens derived from P22 wild type and P22 *int*⁻.

Transductions were performed by infecting log phase cultures of the recipients (containing approximately 10^8 cells per ml, suspended in T_2 buffer) with phage at the appropriate concentration. Transduction mixtures were incubated for 10 minutes at 37° and plated on enriched minimal medium, diluted so as to give of the order of 200 colonies per plate. Where the dilution was large, recipient bacteria were added immediately prior to plating in order to enable haloe production (see below) to be detected. Colonies were counted after 48 hours' incubation, then re-incubated to allow full development of haloes. The total number of colonies counted in each transduction was usually about 1000. Whether or not transductants were lysogens was determined initially by testing for immunity, and for the ability to release transducing phage; prior to testing, the transductants were purified by streaking twice for single colonies onto minimal medium. Later it was found that transductants carrying P22*pro* prophages were "haloed" (see part 3 of RESULTS), and these haloes were used in the scoring. The proportions of *pro*⁻ cells in *pro*+ colonies were estimated by suspending the colonies in T_2 buffer, plating for single colonies on minimal medium + proline, and replicating onto minimal medium.

The interpretation of some of the data for P22*pro-1* depends on the assumption that at low multiplicities of infection cells have been singly infected. Since special precautions against superinfection were not taken, and since it has been observed in other systems that very low m.o.i. may be needed to ensure single infection of cells (HOPPE and ROTH 1974), the reasons for assuming single infection ought to be explained. (a) Previously, comparison of transductions by P22*pro-1* in the presence and in the absence of P22 anti-serum had not revealed differences between the number and properties of transductants recovered at low m.o.i. (JESSOP 1972). (b) The fact that the majority of phages in P22*pro-1* lysates are defective in single infection (see part 1 of RESULTS) suggests that the problem of secondary infection at low m.o.i. should not be very great. (c) Experiments reported in part 3 of the RESULTS show, at low m.o.i., a linear decrease of transductants formed by lysogenization with decreasing m.o.i. (Figure 3).

RESULTS

1. *Properties of transducing phages present in lysates of P22*pro-1 *and P22*pro-3

The data already published for P22*pro-1* lysates showed that these contain transducing phages which can form plaques at multiplicities of infection as low as 10^{-6}; furthermore, when lysates were used at comparable multiplicities of infection in transductions, some of the transductants selected were capable of yielding transducing phage. Similar data have since been obtained for P22*pro-3* lysates. Thus, it seems likely that P22*pro-1* and P22*pro-3* lysates contain transducing phages that are effectively non-defective. However, the data obtained from the experiments outlined below show that both lysates contain, in addition to the above-mentioned phages, phages that are defective; though the defective phages comprise the majority class in both lysates, the properties of the P22*pro-1* defective phages are very different from the properties of the P22*pro-3* defective phages.

Thus, while P22*pro-1* lysates contain transducing phages which can form plaques at very low multiplicities of infection, the number of plaques formed by

TABLE 2

*Plaque-assays of P22*pro-1 *and P22*pro-3

Phage lysate	Ratio of input phage to viable bacteria	*am* mutants used in co-infections	No. of plaques formed per ml phage lysate Indicator strain *proA15su*⁻	DB136	DB147	DB5057*pro*⁻	DB5201*pro*⁻
P22*pro-1*	5	—	7×10^8	—	—	—	—
	10^{-3}	—	5×10^7	—	—	—	—
	10^{-4}	—	1.9×10^7	—	—	—	—
	10^{-5}	—	1.3×10^7	7×10^8	1×10^9	7×10^9	7×10^7
	10^{-4}	*amH79*	2×10^9	—	—	—	—
	10^{-4}	*amH80*	2×10^9	—	—	—	—
P22*n-1*	10^{-5}	—	3×10^9	7.1×10^9	1.21×10^9	1.5×10^9	1.4×10^9
P22*pro-3*	10	—	1×10^9	—	—	—	—
	10^{-4}	—	4×10^9	1.2×10^9	1.9×10^{10}	1.7×10^9	1.7×10^9
	10^{-4}	*amH79*	5×10^9	—	—	—	—
	10^{-4}	*amH80*	2×10^{10}	—	—	—	—
P22*n-3*	10^{-5}	—	3×10^9	1.7×10^9	3.8×10^9	2.9×10^9	2.8×10^9

Numbers of p.f.u. were calculated from counts of approximately 1000 plaques, except in the low m.o.i. experiments, without helper phage, when the numbers of plaques were very low; in these only about fifty plaques were counted. The results given are those of a single experiment; however, comparable results were obtained for the phage lysate used, and for other phage lysates, in a number of experiments.

The total numbers of phage particles in P22*pro-1* and P22*pro-3* lysates were estimated from the numbers of plaque-forming particles observed in co-infections with *amH80*.

transducing phages is increased approximately fiftyfold at multiplicities high enough to ensure double infection of the majority of cells (Table 2), suggesting that the lysates contain large numbers of phages that can form plaques only in mixed infection. If this is so, increases in the number of plaques would be expected on co-infection of a *su*⁻ host with P22*pro-1* and *am* mutants, and on plating P22*pro-1* on strains lysogenic for prophage deletions (CHAN and BOTSTEIN 1972). Such increases have indeed been found with the two *am* mutants tested, and with three of the four prophage deletion strains used as indicator (Table 2). It seems likely from all these data that P22*pro-1* and P22*tetR* lysates are comparable.

For P22*pro-3* lysates, the number of plaque-forming transducing phages detectable is independent of the multiplicity of infection, suggesting that these lysates do not contain defective phages which co-operate in mixed infection. However, while co-infection with *amH79*, and plating on prophage deletions DB136 and DB5057, do not affect estimates of numbers of plaque-forming particles, an increase in the number of plaques is found on co-infection with *amH80*, and on plating on DB147 (Table 2), the increase being of the order of tenfold. Thus defective phages are present but, unlike the P22*pro-1* defective phages, their genomes are not complementary. It is not clear from the genetic data if these phages are transducing; the presence of *pro*⁺ cells in plaques on DB147 could not be tested for since the P22*pro* phages fail to transduce this strain, and although in one experiment some of the plaques formed on co-infection of *proA15*

with *amH80* have appeared to contain *pro*$^+$ cells, these cells grew very slowly on minimal medium, and in later experiments were not recoverable at all. The P22*pro* lysate used in the experiments has been found to contain quite a high proportion of *proC* transducing particles (see part 4), but it seems unlikely that these are the defective particles detected on DB147 and in co-infections with *amH80*, since *pro*$^+$ cells have never been recovered from plaques on *proC437*, and furthermore, the total number of *proC* particles in the lysate is lower, not higher, than the total number of *proAB* transducing particles (Table 6).

2. *Phage genes present in P22*pro-1 *and P22*pro-3 *lysates*

Since phages in P22*pro-1* and P22*pro-3* lysates can form plaques in the absence of helper phage, the genes essential for the lytic cycle must be present. However, the phages could be defective for genes concerned with other phage functions—for example, genes concerned with the reductive response. The presence of genes concerned with the lysogenic cycle has been tested for in experiments carried out on complete medium, using the P22*pro* phages, or their non-transducing derivatives.

a) *Frequency of lysogenization*: Approximately the same proportion of cells is lysogenized by the non-transducing derivatives of P22*pro-1* and P22*pro-3* as by P22 wild type (Table 3), suggesting that the *c* genes, which are concerned with the control of the reductive response, are present.

b) *Stability of lysogens*: The segregation of phage-sensitive cells from lysogens carrying P22*pro* transducing phages, or their non-transducing derivatives, was tested for as described in METHODS. The data, presented in Table 4, indicate that the prophage is not stably integrated in these lysogens. The nature of its defect has not been determined; however, the data presented by CHAN and BOTSTEIN (1976) suggest that the attachment site is faulty.

3. *Transduction of* proA15 *and* proAB47 *by P22*pro-1 *and P22*pro-3 *lysates*

Evidence has been presented previously (JESSOP 1972) that transductants derived from *proA15* × P22*pro-1* do not segregate significant numbers of *pro*$^-$ cells; transductants derived from *proAB47* × P22*pro-1* segregate *pro*$^-$ cells when first isolated, but after purification on minimal medium become stable. Com-

TABLE 3

*Frequency of lysogenization by P22*n-1 *and P22*n-3

Phage lysate	Proportion of lysogenized cells
P22*n-1*	47%
P22*n-3*	47%
P22 c_1	0.1%
P22 wild type	49%

Approximating 1000 infected cells were screened for each phage. The non-transducing derivatives of the P22*pro* phages were used because the small plaques formed by the transducing phages were difficult to see on the EMB medium used to distinguish the fermenting strain used as the host from the non-fermenting strain used as the indicator.

TABLE 4

*Stability of lysogens derived from P22*pro *phages, on complete medium*

Phage carried by lysogen	No. lysogenic colonies segregated	No. non-lysogenic colonies segregated
P22*pro-1*	26	81
P22*pro-3*	41	61
P22 wild type	38	1
P22 *int*$^-$	8	23

parable data have since been obtained for transductants derived from *proA15* × P22*pro-3* and from *proAB47* × P22*pro-3*. Thus, although the P22*pro* phages do not integrate normally under non-selective conditions, it seems likely that under selective conditions recombination may occur between the genome of the transducing phage and the genome of the recipient cell, so that the *pro*$^+$ alleles are not readily lost. Several possible recombination events may be envisaged.

1. The recombination system of the host could effect recombination between the *pro* genes of the transducing phage and the *pro-* genes of the host, as in generalized transduction (Figure 2a); this mechanism is referred to as transduction by substitution (OZEKI and IKEDA 1968). Since such transduction could occur whether or not the transducing phage can circularize, transductants could arise from single infection by defective transducing phages. These transductants would not be expected to carry any phage genes.

2. The transducing phage could be integrated into the host genome, to form a complete lysogen; this mechanism is referred to as transduction by lysogenization. The integration could be brought about by site-specific recombination between the attachment site of the transducing phage and the attachment site of the host (Figure 2b). If the transducing phage were non-defective, the trans-

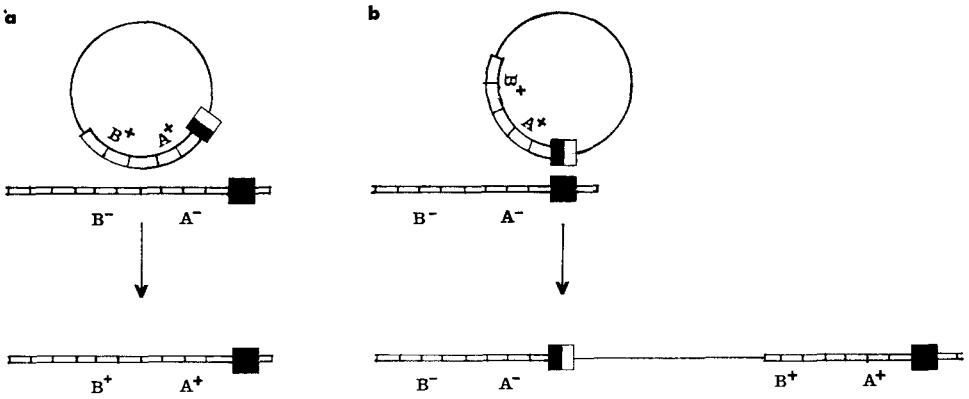

FIGURE 2.—(a) Transduction by substitution; a double crossover causes B$^-$A$^-$ to be replaced by B$^+$A$^+$. (b) Transduction by lysogenization; a single cross-over between the *att* region of the host integrates the transducing phage. The *att* region of the phage is represented as a hybrid, derived from the bacterial and phage attachment sites (CHAN and BOTSTEIN, 1976).

ductants should be immune and should yield phage induction. In the case of defective transducing phages, mixed infections of *pro*$^-$ recipients with differently defective phages, or with defective phages and with P22 wild type as helper phage, could result in lysogenization by the transducing phages, the transductants being expected to have the same properties as transductants derived from lysogenization by non-defective transducing phages. However, in single infections by defective transducing phages lysogenization could only occur if sufficient of the phage genome were lost to enable circularization to occur; transductants derived in this way might be expected to carry some, but not all, phage genes. There is also the possibility that some bacterial DNA (outside the region required for *proAB* transduction) could be lost. If this were so the phage lysate yielded by the transductant should consist of non-defective transducing phages.

Integration of the transducing phage could be brought about by the recombination system of the host causing a single crossover between the *pro* genes of the phage and those of the host. The recovery of such transductants would be expected to be reduced in *rec*$^-$ hosts; however, so far investigation of this possibility has not been straightforward. P22*pro-1* and P22*pro-3* form plaques on *rec*$^-$ strains at a much lower efficiency than they do on *rec*$^+$ strains, indicating that the initial circularization event is more efficient in the presence of the host recombination enzymes; thus transduction frequencies are not an accurate guide to the nature of the mechanism of transduction. Preliminary studies of *pro*$^+$ transductants derived from *pro*$^-$ *rec*$^-$ hosts suggest that such transductants may segregate many more *pro*$^-$ cells than do transductants derived from *pro*$^-$ *rec*$^+$ hosts; however the data are not sufficiently extensive as to warrant conclusions regarding the role of the host recombination enzymes in the integration of the P22*pro* prophages.

a) *Transduction by P22*pro-3 *lysates*: The properties of the majority of transductants derived from *proA15* × P22*pro-3* and *proAB47* × P22*pro-3* transductions are those expected of transductants formed as a result of lysogenization by non-defective transducing phages; that is, cells are immune and yield phage on induction (the phage yielded is usually P22*pro-3*, but may be its non-transducing derivative). The number of transducing particles corresponds approximately to the number of plaque-forming particles (Table 5, columns 3, 4 and 6), and the decrease in the multiplicity of infecting phage to bacteria (m.o.i.) results in a directly proportional decrease in the number of transductants; thus it appears that transduction is effected only by the non-defective transducing phages present in P22*pro-3* lysates. So far, no convincing evidence has been obtained for complete transduction by the defective phages. Thus, although there is a small increase in the number of transductants recovered when P22 wild type is present as helper phage (Table 5), the total number of defective phages is very much greater than the total number of detectable transducing particles. Abortive transductants have not been looked for.

b) *Transduction by P22*pro-1 *lysates*: When *proA15* is transduced by P22*pro-1* lysates at high multiplicities of infection, the majority of the transductants are immune and yield phage on induction (as with transductants derived from

TABLE 5

Transduction of proA15 *and* proAB47 *by P22*pro-1 *and P22*pro-3

Phage lysate	Ratio of input phage to bacteria	Helper Ø	No. plaques formed per ml undiluted phage lysate (on *proA15*)	No. transductants formed per ml undiluted phage lysate			
				proA15 recipient		*proAB47* recipient	
				Transductants formed by lysogenization	Transductants formed by substitution	Transductants formed by lysogenization	Transductants formed by substitution
P22*pro-1*	5	—	7×10^8	8×10^8	1×10^7	1×10^9	0
	10^{-5}	—	1.3×10^7	1.5×10^7	1.9×10^8	1.5×10^7	0
	10^{-5}	P22 wild type	—	*2×10^9		*3×10^9	
P22*pro-3*	5	—	1×10^9	1×10^9	0	1×10^9	0
	10^{-4}	—	4×10^9	1×10^9	0	3×10^8	0
	10^{-4}	P22 wild type	—	*2×10^9		*3×10^9	

* The mechanism of transduction in the presence of helper phage has not been studied.

Each transduction was performed at least three times, with comparable results each time; data from single representative transductions are presented. The numbers given were estimated from counts of about 1000 colonies, with the exception of some of the transductions performed at very low m.o.i., where the numbers were estimated from counts of about 50. The total number of phage particles per undiluted phage suspension was estimated from the numbers of transductants formed on *proA15* in the presence of helper phage.

P22*pro-3*, the phage yielded is usually the transducing phage but may be its non-transducing derivative). The conclusion that the defective phages in P22*pro-1* lysates co-operate in mixed infection to transduce by lysogenization is supported by the observation that the number of phages that transduce by lysogenization corresponds with the number that form plaques at comparable multiplicities (Table 5, columns 3 and 4). When the m.o.i. is decreased, the number of transductants decreases in direct proportion, indicating that the above mechanism is not the only one operating. Many of the transductants formed at low m.o.i. are non-immune and fail to yield phage on induction. Such transductants could either be formed by substitution, or by lysogenization by defective phages which have been enabled to circularize through loss of phage genes. In order to distinguish between these two possibilities transductants were used as indicators in estimates of the number of plaque-forming particles in P22*pro-1* lysates. Out of a total of 64 transductants tested in this way, none has enabled the defective phages to form plaques. Accordingly, it seems likely that the majority of transductants recovered at low m.o.i. are formed by substitution. It appears that P22*pro-1* phages may transduce by lysogenization or by substitution. During the course of the investigation it was noticed that the two kinds of transductant could be distinguished on transduction plates; transducing phage released from the former gives rise to secondary transductants so that the colonies appear "haloed", their appearance contrasting sharply with that of the clean-edged colonies formed by substitution. This observation has facilitated the interpretation of data from transductions carried out at different multiplicities. Between m.o.i. 10–10^{-3} the number of transductants formed by lysogenization is reduced at the rate expected if such transductants require double infection; however, between m.o.i. 10^{-4}–10^{-6}, when few or no such transductants are expected, they continue to be formed, but the numbers now decrease linearly with decreasing m.o.i. The data are shown in Figure 3, where the decrease in numbers of transductants formed by lysogenization is compared with the decrease in the number of transductants formed by substitution. These data suggest that there are phages in P22*pro-1* lysates which can transduce *proA15* by lysogenization in single infection; they agree with the earlier data (JESSOP 1972), which showed that P22*pro-1* could be recovered from *proAB47* transduced at m.o.i. 10^{-7}, and from plaques on plates carrying only 300 phage particles.

When *proAB47* is the recipient, only transductants formed by lysogenization are recovered, the class of transductants formed by substitution being lost. At high m.o.i. the numbers suggest that the defective phages have co-operated (Table 5, column 6). As mentioned above, the transductants recovered at very low m.o.i. are interpreted as resulting from lysogenization by non-defective transducing phages.

4. *Transduction of* proC437 *by P22*pro *phages*

While transduction of *proC* had not previously been observed to occur at unusually high frequencies, the lysates of P22*pro-1* and P22*pro-3* used in the current series of experiments were found to contain unexpectedly high propor-

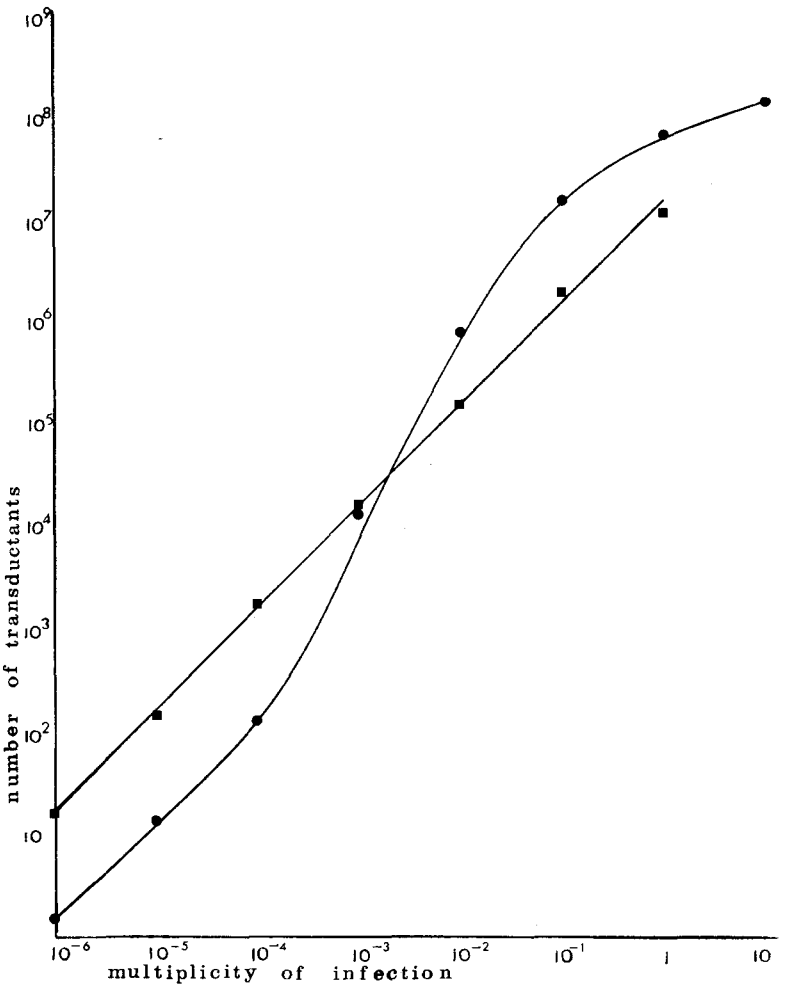

FIGURE 3.—Variation in the number of transductants formed per ml undiluted P22*pro-1* lysate, with variation in the multiplicity of infection.

● transductants formed by lysogenization.

■ transductants formed by substitution.

See Results part 3, and Fig. 2, for explanation. Transductions were performed as described in the METHODS. The positions of the majority of the points were estimated from counts of approximately 1000 colonies.

tions of *proC* transducing particles. A preliminary survey of several independently isolated P22*pro* lysates has indicated that transduction of *proC* at high frequencies (though not as high as transduction of the *proAB* region) may be usual (Table 6).

Lysates showing high frequency of transduction of *proC* have been obtained by induction of lysogens of the *pro* point mutant *proA15*, but not by induction of lysogens of *proAB47* (the P22*pro-1* lysate previously described had been isolated from a *proAB47* lysogen). Since in *proA15* the prophage presumably integrates

TABLE 6

Transductants of proC

Phage lysate	M.o.i.	Helper phage	No. transductants per 10^9 phage particles
P22 wild type	10^{-4}	—	1×10^3
P22*pro-1* (a)	10^{-4}	—	1×10^6
P22*pro-1* (b)	10^{-4}	—	2×10^5
P22*pro-1* (c)	5	—	1×10^6
	10^{-4}	—	3×10^6
	10^{-4}	P22 wild type	3×10^6
P22*pro-3* (a)	10^{-4}	—	1×10^5
P22*pro-3* (b)	5	—	2×10^5
	10^{-4}	—	3×10^5
	10^{-4}	P22 wild type	5×10^5

The P22*pro* lysates were isolated from transductants recovered from different *proA15* × P22*pro* transductions. The P22 wild type lysate used as control was isolated from a lysogen carrying P22 wild type. The P22 wild type helper phage was prepared by lytic infection of *proC437* by P22 wild type.

The number of phage particles in the P22*pro* lysates were estimated from the number of plaque-forming particles detectable at high m.o.i., in the presence of *amH80* (see Table 2).

at *ataA*, which is situated between *proA* and *proC*, while in *proAB47* integration presumably occurs elsewhere in the genome, it seems reasonable to suppose that faulty excision of the prophage gives rise to the *proC* transducing particles. Transduction of *proC* by two of the lysates has been carried out in the presence of P22 wild-type as helper phage, and at high m.o.i., without any change in the frequency of transduction being observed (Table 6).

5. *Density gradient analyses*

The presence of defective and non-defective transducing phages in the same phage lysate is not predicted by the "insertion" hypothesis. One possible explanation could be that the non-defective phages contain more DNA than the defective phages. P22*pro-1* and P22*pro-3* lysates have both been run several times on CsCl gradients without achieving an unequivocal separation of defective and non-defective phages. However, failure to observe such a difference cannot be taken as meaning definitively that all phage heads in the lysates contain the same amount of DNA.

DISCUSSION

It has been found that P22*pro-1* lysates contain a majority of defective transducing phages, only able to lysogenize or to go through the lytic cycle in mixed infection; these phages seem to be analogous to the P22*tetR* phages described by CHAN and BOTSTEIN (1972) in that they behave as though they carry an insertion of bacterial DNA too large to permit encapsulation of a functional phage genome at maturation. In P22*pro-3* lysates the fact that about 10% of the phages behave like non-defective transducing phages suggests that P22*pro-3* phages carry a smaller insertion of bacterial DNA.

P22*pro-1* differs from P22*tetR* in that its lysates contain, in addition to defective phages, a small proportion (1% or less) of transducing phages which can form plaques, or transduce by lysogenization, in single infection. These phages are referred to as non-defective transducing phages. P22*pro-3* lysates also are heterogeneous for defective and non-defective transducing phages, but the defective phages differ from those in P22*pro-1* lysates in that they fail to co-operate in mixed infection; it would appear that they form a single class, or possibly several classes but too few to enable mixed infections to be detectable.

The presence of effectively non-defective transducing phages in P22*pro-1* lysates is surprising. Since transductants derived from these phages in single infection yield lysates which contain a majority of defective transducing phages, the non-defective phages cannot be regarded as having lost sufficient bacterial DNA, or non-essential phage genes, to render them non-defective; if this had happened, all their progeny should be non-defective. Several speculations can be considered: (a) a few phage heads could contain more DNA than usual. The fact that non-defective transducing phages have not been found in P22*tetR* lysates could be explained by the fact that the 20% *tetR* insertion is considerably longer than the net 12.6% insertion found in P22*pro-1* phages (CHAN and BOTSTEIN 1976). (b) the bacterial genome could contain defective prophages, the genetic material of which is similar enough to that of P22 to enable a few of the defective phages in P22*pro-1* lysates to go through the lytic and reductive cycles. The presence of defective phages in *Salmonella typhimurium* has previously been inferred (YAMAMOTO 1964; YOUNG, HARTMAN and MOUDRIANAKIS 1966; HOPPE and ROTH 1974), the defective phages being thought to package some phage genomes in lysates derived from P22 lysogens. However, the data obtained so far on inactivation of phages in P22*pro-1* lysates by antiserum to P22 wild type (JESSOP 1972) have not suggested that serologically unrelated particles might be present.

In P22*pro-3* lysates it is not obvious from the data whether or not the defective phages are transducing. It is possible that there could be occurring loss of the *pro* genes accompanied by loss of an essential region of phage DNA, though it seems unlikely that such an event should occur at such a high rate as to result in a lysate's consisting of a majority of defective phages. Furthermore, it would seem reasonable to expect a similar event to occur at detectable frequencies in P22*pro-1* lysates.

On the assumption that the defective phages are transducing, several structures for P22*pro-3* phages might be considered. The possibility that the insertion is exactly equivalent in length to the terminal redundancy can be rejected, since this would lead to lysates homogeneous for phages lacking terminal redundancy. A structure comparable to that proposed for P22*pro-1*, but carrying a smaller insertion, seems unlikely in view of the patterns of plating of the defective phages (Table 2). Two further possibilities might be considered: (1) If the insertion were just small enough to permit packaging of a fully functional phage genome, then a situation could be envisaged in which all the phages are physically equivalent, but only some are functional—for example, if at maturation an

operon were split in some genomes but not in others. (2) There could be small variations in the lengths of the DNA packaged, so that some phage heads contain a functional phage genome while others do not. Although this seems in many ways to be the most satisfactory explanation for the data, some points remain unclear. Thus, as mentioned above, the patterns of plating of the defective phages do not support the idea of a structure in which terminal redundancy and a few phage genes are missing from each particle. However, if the small phage particles are missing terminal redundancy only, they might be expected to be assisted in circularization by any structurally normal P22 genome; thus both of the *am* mutants tested should have enabled them to go through the lytic cycle, while P22 wild type should have enabled them to transduce by lysogenization.

The overall conclusion from the genetic data is that P22*pro-1* carries an insertion comparable in size to that carried by P22*tetR*, while P22*pro-3* carries a much smaller insertion. This conclusion has been confirmed by the physical data obtained by CHAN and BOTSTEIN (1976) that demonstrate a net insertion of 12.6% for P22*pro-1*, and a net insertion of 3% for P22*pro-3*.

P22*pro-1* lysates differ from P22*tetR* lysates in that transduction can be effected by lysogenization or by substitution. The defective phages in both lysates can transduce by lysogenization in multiple infection; however, while P22*tetR* defective phages only transduce in single infection if they can lysogenize, the P22*pro-1* defective phages transduce *proA* or *B* point mutants by substitution. This difference presumably reflects the fact that *pro* point mutants carry an extensive region of homology to the *proAB* region brought in by the transducing phage, whereas recipients used in P22*tetR* transductions lack the plasmid carrying the *tetR* region. When *proAB47*, which lacks the *proAB* region, is the recipient in P22*pro-1* transductions, the situation is analogous to that in P22*tetR* transductions and P22*pro-1* transduces only by lysogenization. Lysogens carrying defective prophage, such as would result from transduction by lysogenization by defective phages, have not yet been detected among transductants derived from P22*pro-1*, though no doubt more extensive screening would reveal them. The non-defective phages in P22*pro-1* and P22*pro-3* lysates transduce *proA* or *B* point mutants and the *proAB47* deletion by lysogenization. The failure of the defective phages in P22*pro-3* lysates to transduce either by lysogenization or by substitution suggests in the first instance that these phages are not transducing phages; however, although their failure to lysogenize in the presence of P22 wild type as helper is not easy to explain, their failure to transduce by substitution could be due to the gross 6% insertion being too short to permit pairing. A search for abortive transductants could resolve this question.

Although the transductants formed by lysogenization of *proA* or *B* point mutants by P22*pro* phages do not segregate detectable numbers of phage-sensitive, *pro*$^-$, cells, lysogens derived from the transducing phages under non-selective conditions, and from their non-transducing derivatives, are usually unstable. The exact nature of the integration defect has not been determined. However, if the

phages originated from a faulty excision of the P22 wild-type prophage from the host genome, then they would carry a hybrid attachment site derived from the bacterial and phage attachment sites, and transducing phages carrying such sites have been found to integrate at low efficiency (GUERRINI 1969; WEISBERG and GOTTESMAN 1969). It has previously been suggested that the P22*pro* phages originated during the lytic cycle, rather than during induction of a lysogen. Since the phage lysates had been prepared by the agar layer method, which involves many cycles of growth, it has not been definitively shown that some lysogenization, with subsequent induction, had not occurred. Phage lysates harvested after single growth cycles have not had titers comparable to those of lysates prepared by the agar layer method; thus very extensive screening would be needed to detect the presence of specialized transducing phages. While it seems very possible that some lysogens were formed during preparation of the lysates containing the P22*pro* phages, it also seems feasible that interaction between the phage and bacterial attachment sites occurs during the lytic cycle.

The failure of the P22*pro* phages to transduce DB136 and DB147 at high frequencies is unlikely to be due to failure of the transducing phages to interact with the bacterial genome. Although the fact that these bacterial strains are deleted for the whole of the *proA* gene and for a part of the prophage genome suggests the possibility that the formation of complete lysogens might be abnormal, it would seem reasonable to suppose that lysogenization could occur at an alternative attachment site, as apparently happens when *proAB47* is transduced. The failure to recover transductants from DB136 and DB147 can most reasonably be ascribed to the transducing phages being prevented from initiating the reductive response. It has been suggested that the *immI* region, which is present in the prophage, may offer some immunity, since plaques formed on these strains are very faint (CHAN and BOTSTEIN 1972). It seems likely that the immunity generated by *immI* prevents the establishment of lysogeny.

A surprising property of the P22*pro* lysates used in the experiments is their ability to transduce *proC* at high frequency. It is very probable that these *proC* particles are the result of faulty excision of the prophage; the P22*pro* phages are defective in integration, and it has previously been shown for λ and P22 that *int*$^-$ prophages integrated into the bacterial chromosome tend to excise abnormally (GINGERY and ECHOLS 1968; SMITH 1968). The P22*pro-1* lysate originally tested for *proC* transduction (JESSOP 1972) had been isolated from a *proAB47* transductant, in which the prophage was presumably integrated away from its usual site; thus it is not surprising that this lysate did not contain large numbers of *proC* transducing particles. The nature of the *proC* particles has not been determined, though preliminary studies suggest that they may not be similar to the particles transducing the *proAB* region.

MISS MOIRA HOGG gave willing and able technical assistance. DR. DONALD RITCHIE kindly ran the CsCl gradients. DRS. DAVID BOTSTEIN and RUSSELL CHAN helped the work forward by their interest, gifts of stocks, and communication of unpublished results. DR. RUSSELL CHAN'S critical reading of the manuscript was of great value.

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