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# Transduction by Phage P22 in a Recombination-Deficient Mutant of *Salmonella typhimurium*[1]

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Recombination-deficient (Rec$^-$) mutants of *Salmonella typhimurium* were used to study special transduction by phage P22 in the absence of the usual background of general transduction. Rec$^-$ strains could not be transduced for any marker with a lysate arising by infection. When the same strains were infected with a lysate obtained by induction, low but significant levels of transduction were observed for the *pro A* and *pro B* loci (located to one side of the prophage attachment site), but not for *pro C* (located to the other side of the attachment site), *leu*, or *thy*. Integration-deficient (*int*) phage lysates were unable to transduce the *pro A* and *pro B* loci in Rec$^-$ cells unless *int*$^+$ helper phage were present. These results suggest that although Rec$^-$ cells cannot undergo general transduction, special transduction (by lysogenization) for the *pro A* and *pro B* loci is found. A mixture of *int*$^-$ and *int*$^+$ phage were unable to transduce the *pro C* locus despite the large number of *pro C*-transducing particles present in an *int* lysate. The inability of the *int*$^+$ phage genomes to promote successful lysogenization of *pro C*-transducing particles in Rec$^-$ cells may indicate a requirement for a structural element near the left prophage end for integration of phage P22. Alternatively, the deletion of $c_2$, the structural gene for P22 repressor, from most *pro C*-transducing particles may prevent successful lysogenization by these particles. Finally, the *pro C* locus may lie too far away from the prophage site to be included in special transducing particles containing any phage DNA.

## INTRODUCTION

The transfer of bacterial genes from one bacterial cell to another by means of a phage vector is known to occur in at least two ways, both commonly called *transduction*. In *general transduction*, a small portion of the bacterial genome is enclosed in phage coat (Ikeda and Tomizawa, 1965). Upon infecting a bacterium, this fragment of bacterial DNA may undergo recombination with the cell chromosome so that new genes are incorporated. The recombinational event between host genome and injected bacterial fragment is probably carried out by the same enzyme system responsible for recombination during bacterial conjugation. The only difference between conjugation and general transduction may be the mode of entry of the genetic material. General transducing lysates of phages such as *Salmonella typhimurium* phage P22 (Zinder and Lederberg, 1952) and *Escherichia coli* phage P1 are produced both by infection of a sensitive host and by induction of a lysogen.

In *special transduction*, only those bacterial markers adjacent to the prophage attachment site can be transduced. Special transducing particles contain bacterial markers associated with a portion of the phage genome. These particles are usually deleted in phage genes on the side opposite to the incorporated bacterial DNA. When such a phage particle infects another bacterial cell lysogenization may occur; the transduced

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bacterial fragment will thereupon be integrated into the host genome along with the phage DNA. Special transduction may therefore be considered "transduction by lysogenization" (Luria *et al.*, 1960).[3] Transducing lysates of special transducing phages such as λ are formed only by induction of a lysogen (Morse, *et al.*, 1956a). The mechanisms of incorporation of bacterial fragments in general and special transduction are therefore probably different: General transduction uses the bacterial recombination system while special transduction involves the integration of bacterial markers during lysogenization.

Unlike the general transducing phage P1 for which no unique chromosomal attachment site has been found, phage P22 is integrated within the proline region of the *S. typhimurium* chromosome (Smith and Levine, 1967; Smith, 1968). The *pro A* and *pro B* loci map to one end of the prophage attachment site, and *pro C* to the other (Fig. 1). Thus phage P22 may be expected to transduce these genes by the special transduction mechanism in addition to its general transducing activity for these and other *S. typhimurium* genes. Smith-Keary (1966) has obtained evidence for special transduction of the proline region by induced lysates of phage P22.

Phage P22 thus appears to possess both general and special transducing activity for the proline region of *S. typhimurium*. It is difficult, however, to study special transduction by phage P22 in the presence of a background of general transduction of the same genes. A novel way to distinguish between the mechanisms of incorporation in general and special transduction by phage P22 is to use as a recipient a mutant bacterial strain that is defective in the bacterial recombination system but allows prophage integration. In such a mutant, general transduction would not be expected to occur, while special transduction would still be possible. The isolation and characterization of such a *S. typhimurium* Rec⁻ mutant has recently been described (Wing *et al.*, 1968). Although bacterial recombination is markedly depressed in this strain, prophage integration occurs at only a moderately reduced frequency (Wing, 1968).

Auxotrophic derivatives of this Rec⁻ strain were used as recipients in transduction experiments. The results show that no bacterial markers other than in the proline region can be transduced into Rec⁻ strains. That is, general transduction does not occur. The *pro A* and *pro B* genes are transduced by lysates obtained by induction, but the *pro C* gene is not. The finding of *pro A* and *pro B* transduction into Rec⁻ strains confirms the ability of phage P22 to carry out special transduction. The finding that *pro C* transduction does not occur suggests that a region on the *pro C* side of the prophage map may be required for integration of phage P22.

## MATERIALS AND METHODS

*Bacterial and phage strains.* Mutant strains of *S. typhimurium* LT-2 used were *leu 197; thy 90; pro A15; pro B31; pro C90,* all from the collection of Demerec; Hfr A *pur C7 str A* (=su 576 of Sanderson and Demerec, 1965); Hfr B2 *met A22* (=su 354 of Sanderson and Demerec, 1965); *rec leu 197* (Wing *et al.*, 1968); and derivatives of *rec leu 197* carrying one of the following nutritional markers: *thy 90, pro A15, pro B31,* and *pro C90.* The isolation and characterization of such Rec⁻ strains has been described (Wing *et al.*, 1968).

The phage strain employed for most transduction experiments was wild-type phage P22 from the collection of M. Levine. An integration-deficient phage P22 mutant, *int 4* (previously called *L4,* Smith, 1968), was used to measure transduction in the absence of prophage integration.

*Media.* L broth, soft agar for top layers, buffered saline (Levine, 1957), and tryptone agar (Wing *et al.*, 1968) have all been previously described. Minimal agar contains 20 g of Difco bacto-agar, 10 ml of 40% glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid, 10 g of $KH_2PO_4$, and 3.5 g of $NaNH_4 \cdot H_2O$ in 1 liter of distilled water. Minimal soft agar has the same composition as minimal agar except

[3] Lysates of phage λ can transduce markers adjacent to the prophage attachment site by "substitution" as well as by "addition," the former process probably involving the bacterial recombination system whereas the latter entails lysogenization by the transducing phage (Morse *et al.*, 1956b; Kayajanian, personal communication).

that only 7 g of Difco bacto-agar is used per liter.

*Preparation of transducing lysates.* Transducing lysates were prepared by infection of sensitive cells and by induction of lysogens. Infected lysates were prepared as follows: Low multiplicities (0.5–1.5) of wild-type phage particles were used to infect log phase cells in L broth at 37°. Chloroform was added at 90 min to lyse the cells. Cell debris was removed by centrifugation in a Servall SS34 rotor at 8000 rpm (=7710 *g*) for 10 min. The phage were concentrated by centrifugation of the supernatant at 17,000 rpm (34,800 *g*) for 1 hour. The pellet was resuspended in a small volume of buffered saline, centrifuged again at 8000 rpm to remove any remaining cell debris, and then assayed. A drop of chloroform was added to prevent bacterial growth.

To obtain transducing phage by induction, a lysogenic strain was grown to log phase in L broth. The cells were sedimented and resuspended in buffered saline, exposed to an inducing dose of ultraviolet (about 300 ergs/mm$^2$), and diluted into L broth for further incubation at 30° until the culture cleared. Chloroform was added and preparation of the phage stock then followed the steps outlined above. A lysogen for mutant *int 4* was kindly supplied by Dr. H. O. Smith, who obtained it by mixed infection with *int 4* and normal phage particles (Smith, 1968).

*Transduction.* Log-phase cells in L broth were infected with the transducing lysate at multiplicities of 5–50 phage particles per cell. After a 10-min adsorption period, the infected cells were diluted and layered onto minimal agar plates using minimal soft agar. The plates were incubated either at 37° for 2 days or at 25° for 3 days. Rec⁻ colonies required an additional day of incubation because of their slower growth rate (Wing *et al.*, 1968).

In order to detect the very low frequencies of transduction observed in Rec⁻ strains, 0.9 ml of infected cells in L broth were plated onto minimal agar plates. The large amount of L broth resulted in heavy background growth of auxotrophic cells, but the prototrophic transductants easily outgrew them. Since approximately $10^8$ cells, infected with a multiplicity of about 20 phage, were plated, a single prototrophic colony on the plate represented a frequency of transduction of $5 \times 10^{-10}$ per infecting phage. A transduction frequency of about $1 \times 10^{-9}$ was thus considered the limit of detection of the method.

## RESULTS

In the experiments described below, transduction of the *leu* locus, which lies 6 min from the phage P22 attachment site, and the *thy* locus, located 59 min from the attachment site, were used as measures of general transduction.

### *Transduction of Auxotrophic Rec⁺ and Rec⁻ Strains with a Wild-Type Phage P22 Lysate Obtained by Infection*

*Rec⁺ leu 197*, *rec⁺ pro A15*, *rec⁺ pro B31*, and *rec⁺ pro C90* strains were infected with lysates of wild-type phage P22 obtained by infection. Frequencies of transduction of about $10^{-6}$ per infecting phage particle were observed (Table 1).

Similar transduction experiments were carried out on Rec⁻ strains containing the same auxotrophic markers as the Rec⁺ strains above. The data of Table 1 show that no transductants were recovered from any of these infections. A level of transduction of at least $10^{-9}$ transductants per infecting phage would have been detected.

### *Transduction of Auxotrophic Rec⁺ and Rec⁻ Strains with Wild-Type Lysates Obtained by Induction.*

Rec⁺ and Rec⁻ strains carrying the *leu 197*, *thy 90*, *pro A15*, *pro B31*, or *pro C90*

TABLE 1

TRANSDUCTION OF Rec⁺ AND Rec⁻ STRAINS BY A WILD-TYPE LYSATE OBTAINED BY INFECTION

| Bacterial locus | Frequency of transduction[a] | |
|---|---|---|
| | Rec⁺ | Rec⁻ |
| *leu 197* | $5.5 \times 10^{-7}$ | $<6.7 \times 10^{-10}$ |
| *pro A15* | $1.3 \times 10^{-6}$ | $<6.4 \times 10^{-10}$ |
| *pro B31* | $1.1 \times 10^{-6}$ | $<6.4 \times 10^{-10}$ |
| *pro C90* | $5.2 \times 10^{-7}$ | $<6.7 \times 10^{-10}$ |

[a] Transductants per plaque-forming unit in the infecting lysate.

TABLE 2

TRANSDUCTION OF Rec$^+$ AND Rec$^-$ STRAINS BY A WILD-TYPE LYSATE OBTAINED BY INDUCTION

| Bacterial locus | Frequency of transduction[a] | |
|---|---|---|
| | Rec$^+$ | Rec$^-$ |
| *leu 197* | $5.3 \times 10^{-6}$ | $<7.7 \times 10^{-10}$ |
| *thy 90* | $5.7 \times 10^{-6}$ | $<7.7 \times 10^{-10}$ |
| *pro A15* | $3.1 \times 10^{-5}$ | $6.5 \times 10^{-8}$ |
| *pro B31* | $3.8 \times 10^{-5}$ | $2.1 \times 10^{-8}$ |
| *pro C90* | $3.0 \times 10^{-5}$ | $<7.7 \times 10^{-10}$ |

[a] Transductants per plaque-forming unit in the infecting lysate.

markers were infected with a wild-type lysate obtained by induction. In the Rec$^+$ strains (Table 2), transduction frequencies were generally higher than when lysates obtained by infection were used. In the Rec$^-$ strains (Table 2), no transduction of the *pro C*, *leu 197*, or *thy 90* loci was observed. Transduction levels for the *pro A* and *pro B* loci were about 1000-fold less than for the homologous Rec$^+$ strains, but were clearly above the background level observed with *pro C*, *leu 197*, and *thy 90*. The finding that Rec$^-$ auxotrophic strains could be transduced for the *pro A* and *pro B* loci with lysates obtained by induction of lysogens, but not with lysates obtained by infection, suggests that transduction by integration is taking place.

*Transduction of Auxotrophic Rec$^+$ and Rec$^-$ Strains with Mutant int Lysates*

If transduction of *rec pro A* and *rec pro B* strains can occur only by an integration process, then phages that cannot integrate should be unable to transduce these strains. To test this, several Rec$^-$ and control Rec$^+$ strains were infected with an *int 4* lysate prepared by induction. Table 3 shows that an *int 4* lysate cannot transduce any Rec$^-$ strain, including *pro A* and *pro B*, confirming this expectation. Smith (1968) observed that the *int* function can be complemented *in trans*, so that mixed infection with *int* and wild-type phages can lead to integration of the *int* phage. Infection of Rec$^-$ strains with a mixture of *int* and wild-type phages, neither of which alone can transduce these strains, would then be expected to lead to transduction for *pro A* and *pro B*. Indeed this was the observed result (Table 3). Similar frequencies of transduction were obtained with the mixture of induced *int* lysate and wild-type phages obtained by infection as were found with a wild-type lysate obtained by induction (Table 2). Several Rec$^-$ *pro A* and *pro B* transductants were purified and, as expected, were found to be lysogenic for the *int* phage.

Induced *int* lysates consist largely of defective particles whose genetic content is composed of a portion of the phage genome and a section of bacterial DNA on the *pro C* side of the prophage (Smith, 1968). These particles account for the greatly increased transduction of the *rec$^+$ pro C* strain observed with *int* lysates (Smith, 1968). This is confirmed in Table 3, in which the frequency of *rec$^+$ pro C* transduction by the *int 4* lysate is 600-fold greater than transduction by the wild-type lysate. Clearly the *int 4* lysate contains a high number of *pro C*-transducing particles. Yet, surprisingly, no *pro C* transduction occurs in the Rec$^-$ strain with this lysate, suggesting that the *pro C*-transducing particles are incapable of transducing by integration. Even the addition of wild-type

TABLE 3

TRANSDUCTION OF Rec$^+$ AND Rec$^-$ STRAINS BY AN *int 4* LYSATE OBTAINED BY INDUCTION AND BY A MIXTURE OF *int 4* AND WILD-TYPE PHAGE

| Bacterial strain | Lysate[a] | | |
|---|---|---|---|
| | int 4 (induced)[b] | wild type (inf.)[c] | int 4 + w.t.[d] |
| Rec$^+$: | | | |
| *pro A15* | $3.2\times10^{-6}$ | $2.7\times10^{-6}$ | $2.3\times10^{-5}$ |
| *pro B31* | $2.6\times10^{-5}$ | $8.6\times10^{-7}$ | $5.5\times10^{-5}$ |
| *pro C90* | $2.5\times10^{-4}$ | $5.2\times10^{-7}$ | $3.1\times10^{-4}$ |
| Rec$^-$: | | | |
| *pro A15* | $9.0\times10^{-10}$ | $9.0\times10^{-10}$ | $9.3\times10^{-8}$ |
| *pro B31* | $9.0\times10^{-10}$ | $9.0\times10^{-10}$ | $1.8\times10^{-8}$ |
| *pro C90* | $9.0\times10^{-10}$ | $9.0\times10^{-10}$ | $9.0\times10^{-10}$ |

[a] Transduction frequencies for each lysate are given as the number of transductants per plaque-forming unit in the lysate.

[b] An *int* lysate obtained by induction of a lysogen.

[c] A wild-type lysate obtained by infection.

[d] A mixture of the *int* and wild-type lysates in the approximate ratio 5 *int*:1 wild type.

phage (Table 3) cannot help them to integrate.

## DISCUSSION

It would appear that the transduction observed in Rec⁻ strains is of the special type, occurring only when prophage integration is possible. This is strongly suggested by the following findings: (1) Only lysates obtained by induction can carry out transduction of Rec⁻ strains. (2) Transduction is observed only for the *pro A* and *pro B* markers adjacent to the prophage attachment site. (3) Transduction for the *pro A* and *pro B* markers is found under conditions when the bacterial recombination system, presumably required for general transduction, is defective. (4) Phage that are integration-deficient cannot transduce Rec⁻ strains unless wild-type phage are also present to complement the *int* function.

Results obtained with *E. coli* Rec⁻ strains are in agreement with the above findings. No transduction by the general transducing phage P1 is found (Hertman and Luria, 1967). However, transduction of the *bio* locus (adjacent to the prophage attachment site) by some of the special transducing strains of phage λ is observed, although at only 7% the normal frequency (Manly, Signer, and Radding, in press).

An unexpected finding was the absence of *pro C* transduction in the Rec⁻ strain by an *int* lysate even in the presence of wild-type helper phage, despite the large number of *pro C*-transducing particles in the lysate. The *pro C*-transducing particles are apparently unable to integrate. Thus incorporation of *pro C* transducing fragments in Rec⁺ bacteria probably occurs by the general transduction mechanism, i.e., the bacterial recombination system. The consistently higher transduction of the *pro C* locus observed in the Rec⁺ strain infected with an *int* lysate as compared with phage *int*⁺ infection (Smith, 1968) may be due simply to the increased number of *pro C* fragments present in such a lysate. The mechanism of formation of the *pro C*-transducing particles found in *int* lysates is not yet understood.

A large proportion of defective particles in an *int* lysate lack a portion of the phage genome on the *pro A* side of the prophage (Smith, 1968). Several phage loci, including *int*, map in this area (Smith, 1968; see Fig. 1). A wild-type phage lysate contains many normal particles that can complement missing functions in the *pro C*-transducing genomes. The failure of these normal phage to help the *pro C* particles to transduce the Rec⁻ recipient suggests that a structural element necessary for integration, rather than simply a functional element, may be missing from these transducing genomes. The *pro A* end of prophage P22 may be structurally necessary for integration. This end may be analogous to the λ *b2* region (Kellenberger *et al.*, 1961), the presence of which is also required for prohage integration (Zichichi and Kellenberger, 1963; Campbell, 1965) and which is located close to the λ *int* locus (Zissler, 1967; Gingery and Echols, 1967).

An alternative explanation for the failure to recover Rec⁻ *pro C* transductants could lie in the absence of critical phage genes from the *pro C* transducing particles. The majority of the particles in an *int* lysate are deleted in the $c_2$ region (Smith, 1968), which maps close to the *pro A* end of the prophage genome (Smith and Levine, 1965; Smith, 1968; see Fig. 1). The $c_2$ gene is the structural gene for phage P22 repressor and is required for the initiation and maintenance of lysogeny (Levine, 1957; Levine and Smith, 1964). *Pro C* transducing particles might therefore be unable to initiate and maintain lysogeny, even in the presence of $c^+$ helper phage. *Pro C* transduction could thus occur only by the general recombination mechanism and would be observed only in Rec⁺ recipients.

Yet another possibility for the absence of *pro C* transduction in the Rec⁻ strain is that the *pro C* locus may lie too far from the prophage attachment site to be incorporated into a special transducing particle. The findings of Smith (1968) suggest that an *int*

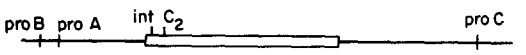


FIG. 1. Location of prophage P22 within the proline region of *Salmonella typhimurium*.

lysate contains a large number of defective particles consisting partly of bacterial DNA from the *pro C* side and partly of phage DNA from the prophage end proximal to *pro C*. However, it has not been possible to demonstrate rigorously that these defective particles are responsible for the high level of *pro C* transduction observed, although this is the most likely hypothesis. The possibility remains that the *pro C* locus is sufficiently far from the prophage attachment site so that particles carrying this marker contain no phage DNA at all and therefore are unable to transduce by the prophage integration mechanism.

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## REFERENCES

CAMPBELL, A. (1965). The steric effect in lysogenization by bacteriophage lambda. II. Chromosomal attachment of the $b_2$ mutant. *Virology* **27**, 340–345.

GINGERY, R., and ECHOLS, H. (1967). Mutants of bacteriophage λ unable to integrate into the host chromosome. *Proc. Natl. Acad. Sci. U.S.* **58**, 1507–1514.

HERTMAN, I., and LURIA, S. E. (1967). Transduction studies on the role of the $rec^+$ gene in the ultraviolet induction of prophage lambda. *J. Mol. Biol.* **23**, 117–133.

IKEDA, N., and TOMIZAWA, J. (1965). Transducing fragments in generalized transduction by phage P1. I. Molecular origin of the fragments. *J. Mol. Biol.* **14**, 85–109.

KELLENBERGER, G., ZICHICHI, M. L., and WEIGLE, J. (1961). A mutation affecting the DNA content of bacteriophage lambda and its lysogenization properties. *J. Mol. Biol.* **3**, 399–408.

LEVINE, M. (1957). Mutations in the temperate phage P22 and lysogeny in *Salmonella*. *Virology* **3**, 22–41.

LEVINE, M., and SMITH, H. O. (1964). Sequential gene action in the establishment of lysogeny. *Science* **146**, 1581–1582.

LURIA, S. E., ADAMS, J. N., and TING, R. C. (1960). Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology* **12**, 348–390.

MORSE, M. L., LEDERBERG, E. M., and LEDERBERG, J. (1956a). Transduction in *Escherichia coli* K-12. *Genetics* **41**, 142–156.

MORSE, M. L., LEDERBERG, E. M., and LEDERBERG J. (1956b). Transductional heterogenotes in *Escherichia coli*. *Genetics* **41**, 758–779.

ROTHMAN, J. L. (1965). Transduction studies on the relation between prophage and the host chromosome. *J. Mol. Biol.* **12**, 892–912.

SANDERSON, K. E., and DEMEREC, M. (1965). The linkage map of *Salmonella typhimurium*. *Genetics* **51**, 897–913.

SMITH, H. O. (1968). Defective phage formation by lysogens of integration-deficient phage P22 mutants. *Virology* **34**, 203–223.

SMITH, H. O., and LEVINE, M. (1965). Gene order in prophage P22. *Virology* **27**, 229–231.

SMITH, H. O., and LEVINE, M. (1967). A phage P22 gene controlling the integration of prophage. *Virology* **31**, 207–216.

SMITH-KEARY, P. F. (1966). Restricted transduction by bacteriophage P22 in *Salmonella typhimurium*. *Genet. Res.* **8**, 73–82.

WING, J. P. (1968). The integration and induction of phage P22 in a recombination-deficient mutant of *Salmonella typhimurium*. *J. Virol.* **2**, 702–709.

WING, J. P., LEVINE, M., and SMITH, H. O. (1968). A recombination-deficient mutant of *Salmonella typhimurium*. *J. Bacteriol.* **95**, 1828–1834.

ZICHICHI, M. L., and KELLENBERGER, G. (1963). Two distinct functions in the lysogenization process: the repression of phage multiplication and the incorporation of the prophage in the bacterial genome. *Virology* **19**, 450–460.

ZINDER, N., and LEDERBERG, J. (1952). Genetic exchange in *Salmonella*. *J. Bacteriol.* **64**, 679–699.

ZISSLER, J. (1967). Integration-negative (*int*) mutants of phage λ. *Virology* **31**, 189.