Genet. Res., Camb. (1966), **8**, *pp.* 73–82
Printed in Great Britain

Restricted trandsuction by bacteriophage P22 in *Salmonella typhimurium*

By P. F. SMITH-KEARY

Department of Genetics, Trinity College, Dublin

(*Received* 25 *February* 1966)

1. INTRODUCTION

Transduction, the transfer of bacterial characters by a phage vector, appears to involve two types of process. In general transduction, exemplified by phage P22 in *Salmonella typhimurium* (Lederberg *et al.*, 1951; Zinder & Lederberg, 1952) and by phage P1 in *Escherichia coli* (Lennox, 1955), the phage particles can transfer any character of a donor strain to a suitable recipient. Phage produced by spontaneous lysis is effective in transduction, and provided that a low ratio of phage to bacteria (multiplicity of infection, MOI) is used to minimize multiple infection, the transductants are normally phage-sensitive, both in the P1–*Escherichia* (Adams & Luria, 1958) and P22–*Salmonella* (Starlinger, 1958; Zinder, 1959) systems. The phages involved in general transduction do not appear to map exclusively at a single prophage locus. In restricted, or special, transduction, first observed with phage λ in *E. coli* (Morse, Lederberg & Lederberg, 1956*a*, 1956*b*) transduction is restricted to a small cluster of genes, the *gal* (galactose) region adjacent to the specific λ prophage locus on the bacterial chromosome. Infection of a gal^- recipient with λ obtained by the induction of a λ lysogenic gal^+ strain results in the formation of gal^+/gal^- partial diploids (heterogenotes) in which the gal^+ segment of the donor chromosome persists associated with, and replacing a part of, a phage genome (exogenote) (Arber, Kellenberger & Weigle, 1957; Campbell, 1958). In the presence of a normal λ prophage the induction of a heterogenote yields a phage preparation which has a very high efficiency for effecting transduction to gal^+ (high frequency transducing or HFT lysate).

In this paper an apparently defective variant of P22 is described, which specifically transduces the proline region of *S. typhimurium* at high frequency. The similarities of this system with λ-mediated transduction in *E. coli* and with the P1-mediated transduction of the *lac* (lactose-utilization) region in *E. coli* and *Shigella dysenteriae* (Luria, Adams & Ting, 1960) will be discussed.

2. MATERIALS AND METHODS

The *pro-401* mutant (Smith-Keary & Dawson, 1964) and the wild-type stocks were of the LT2 strain of *Salmonella typhimurium*. Phage P22 was used as the vector and transductants were selected by plating on enriched minimal medium (EMM).

The media used and the methods of preparing phage and effecting transduction were those previously described (Smith-Keary, 1960).

3. RESULTS

When *pro-401* is infected with phage P22 prepared by the lytic infection of a sensitive wild-type donor and plated on medium selecting for proline-independence, a proportion of the wild-type transductants have characteristic haloes of several hundred small (up to 2 mm. diameter) satellite colonies. The number of satellites, and hence the size of the halo, and the size of any individual satellite increases with the duration of incubation. The satellite colonies (S transductants) and the bacteria in them are phenotypically wild-type and are apparently a consequence of the lytic production of transducing phage by the primary transductant (phage-liberating, PL transductant) which diffuses across the medium and in turn transforms *pro-401* bacteria in the background to wild-type.

When a small number of wild-type bacteria from a phage-liberating transductant are mixed with a large excess of either sensitive or lysogenic *pro-401* and plated on selective medium, a high proportion of the wild-type colonies which grow are satellited; this proportion is higher with a lysogenic than with a sensitive *pro-401* indicator. No satellites were observed when a normally lysogenic wild-type strain was similarly plated with *pro-401* (Table 1).

Table 1. *Frequency of satellited colonies on EMM plates spread with about* 4×10^7 pro-401 *bacteria and with a dilution of either lysogenic wild-type bacteria or cells from a satellited (PL) transductant*

			Colonies scored on indicator strain			
			Sensitive *pro-401*		Lysogenic *pro-401*	
WT cells plated from	Experiment	Expected number of WT colonies	Total	With satellites	Total	With satellites
WT lysogenic	A	416	448	0		
	B	82	76	0	65	0
PL transductant	A	193	204	190		
	B	44	34	16	34	28
	C	110	94	37	126	66

These results are in agreement with the hypothesis that the satellited PL transductants liberate transducing phage, whereas normal lysogens and non-satellited transductants do not. This interpretation was confirmed in the following way.

Inocula were taken from three wild-type lysogenic colonies, three non-satellited (N) transductants and from three satellited (PL) transductants, shaken in broth and added to sloppy agar layer plates without the addition of further phage. Any phage released by spontaneous lysis was harvested from the sloppy agar in the usual way (Smith-Keary, 1960).

Each preparation was used to infect *pro-401* and proline-independent transductants were selected on enriched minimal medium. Each of the suspensions prepared from the PL transductants yielded an average of about 500 transductants per plate, whereas those prepared from the N transductants, or from the wild-type stable lysogens, gave none.

The phage suspensions prepared from the PL transductants have a very high specific transducing titre and yield about 20,000 transductants per 10^8 plaque-forming particles. This can be compared with the corresponding frequency of about 450 transductants per 10^8 plaque-forming particles when wild-type P22 grown on a sensitive donor is used. Over a wide range of multiplicities of infection (0·006 to about 4·0) the yield of transductants from these HFT lysates is proportional to the number of plaque-forming particles. Comparative transduction assays for a wild-type P22 lysate and one of the HFT lysates are set out in Table 2.

Table 2. *Assays of wild-type and HFT phage preparations* (*a*) *and the frequency of wild-type transductants when these preparations are used to infect a sensitive* pro-401 *recipient* (*b*)

(*a*) *Phage assays*

	Turbid plaques	Clear plaques
Wild-type (per ml.)	$1·6 \times 10^{10}$	$0·5 \times 10^9$
HFT (per ml.)	$2·3 \times 10^9$	$1·0 \times 10^9$

(*b*) *Frequency of transductants.* 5×10^7 *bacteria per plate*

Phage preparation	Phage per plate	MOI	Average transductants per plate	Transductants per 10^8 plaque-forming particles
Wild-type	$5·0 \times 10^7$	1·0	209	418
	$2·5 \times 10^7$	0·5	124	496
	$1·25 \times 10^7$	0·25	55	440
	$6·25 \times 10^6$	0·12	26	416
HFT	$2·5 \times 10^6$	0·05	425	17,000
	$1·25 \times 10^6$	0·025	285	22,800
	$6·25 \times 10^5$	0·012	132	21,120
	$3·12 \times 10^5$	0·006	65	20,800

These HFT lysates differ from normal P22 preparations in three ways. Firstly, they transduce the *pro*$^+$ region at a greatly enhanced frequency; secondly, a higher proportion of the transductants are satellited; thirdly, they are unable to transduce, even at low frequency, the leucine, histidine, cystine A, methionine B or tryptophane regions of the genome, and so appear to specifically transduce the proline region.

Assays of the HFT and wild-type P22 preparations (Table 2) show that the HFT lysates contain an unusually high proportion (43%) of particles which produce clear plaques when assayed against a sensitive indicator strain; these appear to be mutants with a reduced capacity for lysogenization. In order to test whether these

particles were responsible for the high frequency proline-specific transduction, three clear and three normal turbid plaques were cut out from the assay plates, shaken in T2 buffer, used to infect a sensitive wild-type donor and the phage harvested by the agar layer method. Each preparation, together with an HFT and a normal wild-type lysate, was used in transductions with *pro-401* as recipient. The preparations were also tested for their ability to transduce the *leu*, *his*, *cysA*, *metB* and *try* regions of the genome. The results of one set of transductions are set out in Table 3. It is

Table 3. *Assays of phage preparations made from clear and turbid plaques* (*a*) *and the frequency of wild-type transductants when these and wild-type and HFT preparations are used to infect a sensitive* pro-401 *recipient* (*b*)

(*a*) *Phage assays*

	Turbid plaques	Clear plaques
Clear plaque preparation (per ml.)	$0{\cdot}5 \times 10^9$	$2{\cdot}13 \times 10^{10}$
Turbid plaque preparation (per ml.)	$2{\cdot}6 \times 10^{10}$	$0{\cdot}4 \times 10^9$

(*b*) *Frequency of transductants.* 5×10^7 *bacteria per plate*

Phage preparation	Phage per plate	MOI	Average transductants per plate	Transductants per 10^8 plaque-forming particles
Wild-type	$1{\cdot}25 \times 10^7$	0·25	103	824
HFT	$2{\cdot}5 \times 10^6$	0·05	270	10,800
Clear plaque	$4{\cdot}0 \times 10^7$	0·8	104	260
Turbid plaque	$1{\cdot}0 \times 10^7$	0·2	65	650

clear that neither type of plaque-forming particle transduces *pro*$^+$ at a frequency higher than the standard wild-type preparation; furthermore, all the preparations, with the exception of the HFT lysate, could transduce all the other regions of the genome for which they were tested. It is most probable therefore that high frequency transduction specific for the proline region is effected by a defective non-plaque-forming variant of P22, analogous to *λdg* (Campbell, 1958, 1960).

In transductions using *pro-401* as recipient, and either wild-type or HFT phage donors, the yield of transductants is linearly proportional to the number of infecting plaque-forming particles at multiplicities of infection of 1 or less. Increasing the MOI also increases the proportion of satellited to non-satellited transductants. It would appear that while helper phage (Luria, Adams & Ting, 1960) is not necessary for the establishment of a wild-type transductant it is necessary for the vegetative replication of the transducing elements carrying the proline region. This interpretation is borne out by a comparison of the proportion of satellited to non-satellited transductants in transductions using sensitive and lysogenic *pro-401* recipients. With a lysogenic recipient, although the number of transductants is reduced by about 60%, the proportion of satellited transductants is increased, particularly at low multiplicities of infection (Table 4).

In the transductions using HFT phage at very low multiplicities of infection, three types of transductant were recognized:

Table 4. *Frequencies of satellited colonies in transductions using low and higher multiplicities of infection*

4×10^7 *sensitive or lysogenic* pro-401 *bacteria per plate*

Phage donor	MOI	*pro-401* recipient	Transductants on five plates: Total	With satellites	Without satellites
Wild-type	0·5	Sensitive	359	10	349
Wild-type	0·5	Lysogenic	88	20	68
HFT	0·004	Sensitive	390	30	360
HFT	0·004	Lysogenic	124	57	67
HFT*	0·5	Sensitive	320	95	215
HFT*	0·5	Lysogenic	141	75	66

* Transduction mixture diluted 1:80 prior to plating.

Satellited (PL) transductants. These are resistant to P22 and on streaking segregate phage-sensitive proline-requiring clones at low frequency and phage-resistant proline-requiring clones at higher frequency.

Non-satellited (N) transductants sensitive to P22. These are stable and do not segregate proline-requiring clones.

Non-satellited transductants resistant to P22. These do not release free phage but frequently segregate phage-sensitive proline-requiring clones.

At higher multiplicities of infection (over about 0·5) no non-satellited phage-sensitive transductants were recovered and some of the non-satellited phage-resistant transductants segregated proline-requiring normal lysogens.

The results of spotting these transductants against a sensitive and a lysogenic

Table 5. *Frequency of satellited colonies when EMM plates spread with about* 4×10^7 *sensitive or lysogenic* pro-*401 bacteria were spotted with inocula taken from satellited (PL) and non-satellited (N) transductants, from the satellite colonies (S) and from lysogenic wild-type colonies*

Type of colony spotted	*pro-401* indicator strain: P22 sensitive: Colonies spotted	P22 sensitive: Colonies with satellites	P22 lysogenic: Colonies spotted	P22 lysogenic: Colonies with satellites
PL transductants	48	28	48	39
S transductants	51	30	52	44
N transductants				
(*a*) P22 resistant	35	4*	36	18
(*b*) P22 sensitive	21	0	17	1*
WT lysogenic	14	1*	24	2*

* Very few satellites in halo.

https://doi.org/10.1017/S0016672300009927 Published online by Cambridge University Press

pro-401 strain are shown in Table 5. Whereas the PL and S transductants yield a high proportion of satellited colonies on both the sensitive and lysogenic indicators, the non-satellited phage-resistant transductants form satellites at low frequency on the sensitive indicator, but at a much higher frequency on the lysogenic indicator. Satellites are not formed, except at very low frequency, by either the non-satellited phage-sensitive transductants or the wild-type lysogens.

4. DISCUSSION

A reasonable hypothesis to explain these results assumes the existence of defective P22 elements formally analogous to the *λdg* (Arber, Kellenberger & Weigle, 1957; Campbell, 1958) and P1-*dl* (Luria, Adams & Ting, 1960) elements, and is as follows.

'Transduction of the proline region is effected by at least two types of defective phage particles. The first is the defective particle involved in general transduction; it is unable to establish itself as prophage or to replicate, it may carry the proline region (or any other transducible marker) and wild-type transductants result from the integration of part of the transduced fragment into the recipient chromosome. The second also carries the wild-type proline region but can establish itself as prophage at the P22 prophage locus adjacent to the proline A gene (Zinder, 1959; Dubnau & Stocker, 1964; Subbaiah & Stocker, 1964; Smith & Levine, 1965), it carries the P22 immunity determinants, so that the infected cell is immune to super-infection, but lacks the ability to replicate unless a normal prophage is also present; with this defective phage transformation of the phenotype is by lysogenization ("transduction by lysogenization", Luria, Adams & Ting, 1960) and the transduced bacterial genes remain incorporated into the phage genome; the *pro*$^+$ genes are not integrated into the bacterial chromosome and the transductants are *pro*$^+$/*pro*$^-$ heterogenotes.'

Consequently, in transductions carried out at very low multiplicities of infection, type 1 defective particles carrying *pro*$^+$ will produce stable proline-independent phage-sensitive transductants; infection with type 2 defective phage results in transduction by lysogenization producing non-satellited phage-resistant transductants, which, by loss of the exogenote segregate P22-sensitive proline-requiring derivatives. The satellited PL transductants result from double infection with both a type 2 defective and a normal particle; the normal particle supplies the functions deficient in the defective transducing phage, vegetative multiplication can occur, and on lysis both normal and defective transducing phage are released. The released type 2 phage can now infect and transduce by lysogenization the *pro-401* bacteria in the background and so produce satellites. Since the satellited transductants carry a normal prophage, loss of the exogenote will normally produce a proline-requiring stable lysogen. Each of these types of transductant and segregant has been recovered in the experiments described.

The proportion of satellited colonies can be increased either by increasing the multiplicity of infection or by using a lysogenic recipient (Table 4). This is expected if the hypothesis is correct, as raising the multiplicity of infection will increase the

proportion of bacteria doubly infected by a normal and a defective phage, while with lysogenic recipients any cell superinfected by a type 2 defective particle will be potentially capable of releasing HFT phage. Further confirmation comes from the results of spotting inocula taken from the transductants on to sensitive and lysogenic *pro-401* indicators (Table 5); whereas the PL and S transductants produce satellites on both the sensitive and lysogenic indicators, the P22-resistant N transductants only yield a high proportion of satellited colonies on the lysogenic indicator, which provides the normal P22 particles necessary to complement the defective transducing phage.

Satellited transductants and high frequency transduction in *Salmonella* have not been observed in any transductions involving auxotrophic markers outside the proline region. Dubnau & Stocker (1964) have described high frequency transduction of tetracycline resistance by P22 in *S. typhimurium*; in this system the transduced marker remains associated with a prophage, the transductants are frequently defective lysogens and the transduced *tet-r* factor maps at the P22 prophage locus. Dubnau & Stocker (1964) infer the existence of a defective transducing phage which becomes associated with the episomic *tet-r* factor, and which in the presence of normal P22 'helper' phage can be replicated during vegetative multiplication. Their results and inferences closely correspond to those described in this paper. It may be that in *Salmonella* specialized transduction is restricted to markers which can function when incorporated into a defective transducing phage; the transduced proline genes are expressed because they are located at the prophage locus immediately adjacent to the proline region on the recipient chromosome, *tet-r* is expressed because this factor is normally episomic (being part of the R factor) and is therefore not dependent on having a precise chromosomal location for its functioning.

There is also a close similarity between these observations, their explanation and both the λ-*gal* and P1-*dl-lac* systems in *E. coli*. In the λ-*gal* system, the *gal* region of an *E. coli* donor replaces part of a λ genome (Morse *et al.*, 1956*a*, 1956*b*; Arber *et al.*, 1957; Weigle *et al.*, 1959; Campbell, 1960). This defective transducing phage, λ*dg*, cannot replicate, the *gal*$^+$ transductants are defective lysogens and the *gal*$^+$ fragment persists associated with the defective prophage as an exogenote; loss of the exogenote produces a λ-sensitive *gal*$^-$ segregant (Campbell, 1957). Transformation of the phenotype is always, at least in the first instance, by lysogenization. If a heterogenote also lysogenic for a normal λ prophage is induced, normal and λ*dg* particles are released in equal numbers (HFT preparation).

In the P22-proline system the PL transductants appear to be lysogenic for a normal P22 and a defective *pro*$^+$ transducing phage. Unlike λ, which must be induced to yield HFT lysates, these transductants spontaneously release phage with a high transducing titre, although this titre is much lower than with λ HFT lysates. The non-satellited phage-resistant transductants correspond to the *gal*$^+$/*gal*$^-$ heterogenotes, and like them are defectively lysogenic and segregate auxotrophic derivatives.

It is a feature of the P22-proline system that the HFT lysates fail to transduce

markers outside the proline region, even at low frequency. Normal lysates of P22 can transduce almost every marker in the genome and it might be expected that HFT P22 preparations would, in addition to transducing *pro*$^+$ at high frequency, transduce other markers at a frequency compatible with their plaque-forming titre. The absence of such transductants indicates that in strains carrying a prophage at the P22 prophage locus adjacent to the proline region, markers outside this region are not incorporated into transducing phage, or are incorporated at such a low frequency as to be undetectable.

The similarities between the P22-proline system and the transduction of *lac*$^+$ by phage P1 (which like P22 participates in general transduction) in *E. coli* (Luria *et al.*, 1960) are even more striking. In this system Luria has deduced the existence of defective P1 particles, P1-*dl*, which can transfer the *lac* region of *lac*$^+$ *E. coli* into *lac*$^-$ strains of *E. coli* or *Shigella dysenteriae*. With *E. coli* recipients transformation of the phenotype is by integration (1 per 10^5–10^6 plaque-forming particles) and the transductants are stable *lac*$^+$ and either P1-sensitive or normal lysogens. When *S. dysenteriae* is used as recipient the frequency of transudction is much lower (1 per 10^7–10^8 plaque-forming particles), the transductants are generally defective lysogens and segregate sensitive *lac*$^-$ derivatives. Ultra-violet induction of some such *Shigella* transductants yields, after superinfection by non-transducing phage, HFT lysates which with *E. coli* recipients yield up to 1 transductant per 100 plaque-forming particles. The transduction of *lac*$^+$ into *Shigella* is attributed to the presence of defective P1 elements, P1-*dl*, which specifically incorporate the *lac*$^+$ region. On the basis of differences in the pattern of lysogeny, immunity and segregation among the *Shigella lac*$^+$ transductants, Luria has deduced the existence of P1-*dl* elements deficient for different segments of their genome. At one extreme are elements which retain the immunity functions and can become relatively stable prophage; these correspond to the defective P22 particles which yield non-satellited defectively lysogenic transductants. At the other extreme are elements with no immunity function and unable to establish themselves as prophage; these correspond to the P22 particles which result in non-satellited phage-sensitive transductants.

Luria (Luria *et al.*, 1960) interprets the difference between transformation by integration of *lac*$^+$ into *E. coli* recipients, and transformation by lysogenization in *Shigella* recipients as reflecting differences in the genetic homology between the donor and recipient strains in the regions immediately outside the *lac* region itself (Franklin & Luria, 1961); poor homology favours transformation by lysogenization as this is a barrier to recombination. It is likely that the relatively high proportion of proline-independent heterogenotic transductants, and the relatively low frequency of stable transductants in which integration of the *pro*$^+$ region has occurred may also be a consequence of poor homology. The auxotrophy of *pro-401* has been shown to be the consequence of the attachment of a controlling episome to a structurally wild-type locus (Smith-Keary & Dawson, 1964); this controlling episome could act as a barrier to recombination between the recipient *pro-401* chromosome and the transduced *pro*$^+$ fragment thereby favouring the establishment of heterogenotes.

SUMMARY

1. In the transduction *pro-401* (×) + some of the transductants are surrounded by several hundred small wild-type satellite colonies; these transductants spontaneously release phage which transduces *pro-401* to wild-type at high frequency (HFT phage).

2. When the HFT phage is used to infect *pro-401* at very low multiplicities of infection, most of the transductants are defective lysogens and segregate proline-requiring phage-sensitive derivatives; these transductants are apparently heterogenotes. At higher multiplicities of infection, or with lysogenic recipients, a higher proportion of satellited transductants is found.

3. The HFT phage preparations transduce only the proline region of the donor genome.

4. The existence is inferred of a defective P22 particle specifically incorporating the proline region of the *Salmonella* chromosome; these defective particles can establish themselves as prophage and confer immunity upon the infected cell, but are unable to replicate unless a normal prophage is also present. Satellited transductants are lysogenic both for a normal and defective (proline region carrying) phage, and so on lysis release transducing phage.

5. This system is compared with the *λdg-gal* and P1-*dl-lac* systems in *E. coli*.

The author has much pleasure in thanking Mr G. W. P. Dawson for his advice and criticism during this investigation. The reaserch reported herein has been sponsored by the Office, Chief of Research and Development, U.S. Department of the Army, through its European Research Office.

REFERENCES

Adams, J. N. & Luria, S. E. (1958). Transduction by bacteriophage P1. Abnormal phage function of the transducing particles. *Proc. natn. Acad. Sci. U.S.A.* **44**, 590–594.

Arber, W., Kellenberger, G. & Weigle, J. (1957). La défectuosité du phage lambda transducteur. *Schweiz. Z. allg. Path. Bakt.* **20**, 659–665.

Campbell, A. (1957). Transduction and segregation in *Escherichia coli* K-12. *Virology*, **4**, 366–384.

Campbell, A. (1958). The different kinds of transducing particles in the *λ-gal* system. *Cold Spring Harb. Symp. quant. Biol.* **23**, 83–84.

Campbell, A. (1960). On the mechanism of the recombinational event in the formation of transducing phage. *Virology*, **11**, 339–348.

Dubnau, E. & Stocker, B. A. D. (1964). Genetics of plasmids in *Salmonella typhimurium*. *Nature, Lond.* **204**, 1112–1113.

Franklin, N. C. & Luria, S. E. (1961). Transduction by bacteriophage P1 and the properties of the *lac* genetic region in *E. coli* and *S. dysenteriae*. *Virology*, **15**, 299–311.

Lederberg, J., Lederberg, E. M., Zinder, N. D. & Lively, E. R. (1951). Recombination analysis of bacterial heredity. *Cold Spring Harb. Symp. quant. Biol.* **16**, 413–441.

Lennox, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology*, **1**, 190–206.

Luria, S. E., Adams, J. N. & Ting, R. C. (1960). Transduction of lactose-utilising 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. & Lederberg, J. (1956*a*). Transduction in *Escherichia coli* K-12. *Genetics*, **41**, 142–156.

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

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

SMITH-KEARY, P. F. (1960). A suppressor of leucineless in *Salmonella typhimurium*. *Heredity, Lond.*, **14**, 61–71.

SMITH-KEARY, P. F. & DAWSON, G. W. P. (1964). Episomic suppression of phenotype in *Salmonella*. *Genet. Res.* **5**, 269–281.

STARLINGER, P. (1958). Über einen defekt des transduzierenden *Salmonella*-phagen P22 übert ragenen materials. *Z. Naturf.* **14b**, 523–528.

SUBBAIAH, T. V. & STOCKER, B. A. D. (1964). Rough mutants of *Salmonella typhimurium*. (1) Genetics. *Nature, Lond.* **201**, 1298–1299.

WEIGLE, J., MESELSON, M. & PAIGEN, K. (1959). Density alterations associated with transducing ability in the bacteriophage lambda. *J. molec. Biol.* **1**, 379–386.

ZINDER, N. D. (1959). Genetic interaction between bacteriophage and bacteria. In *Perspectives in Virology*, pp. 43–53. New York: Wiley.

ZINDER, N. D. & LEDERBERG, J. (1952). Genetic exchange in *Salmonella*. *J. Bact.* **64**, 679–699.