A Novel Pleiotropic Mutation in *Escherichia coli* K12 which Affects Transduction, Transformation and Rates of Mutation

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SUMMARY

A mutant strain of *Escherichia coli* K12, R2721, has been shown to differ from its parent strain, S491, in four associated phenotypic characters as a result of a single mutation. This strain did not give recombinants with DNA transduced by bacteriophage P1 or bacteriophage Mu, nor transformants after exposure to R factor DNA: lysates of bacteriophage P1 grown on this strain did not appear to contain any transducing particles when tested on normal recipients. Moreover, the reversion rates, both spontaneous and ultraviolet-induced, for two auxotrophic markers were reduced. The frequency of revertants was at least two orders of magnitude lower in cultures of R2721 than in cultures of S491. Many of the rare revertants for one or other of the auxotrophic markers were found to have regained normal reversion frequencies for the other marker and for the capacity to be transduced. In all other respects, recombination in R2721 appeared normal, the frequency of chromosomal mobilization by an F' factor was unaffected and normal yields of recombinants were obtained from matings with Hfr strains. The only circumstance in which transduction of R2721 was observed was when the capacity to ferment galactose was selected and P1 had been grown on a strain carrying *λdgal* when, presumably, integration was effected by the phage-coded gene products. The mutation has been located on the *E. coli* chromosome map between *tonA* and *pro* and has been given the symbol *tdi* (transduction inhibition). Double mutants, (*tdi recA*) and (*tdi recB*), have been isolated and show no unexpected properties.

INTRODUCTION

Lloyd (1971) isolated several mutants of *Escherichia coli* K12, S491, suspected of being defective in the process of genetic recombination. One of these, R2721, appeared to be unable to complete the process of P1-mediated transduction. It was tested for the transduction of an R factor and because it gave no drug-resistant transductants it was put to one side. But since it appeared to be a normal host for P1 there remained the possibility that there was, in this strain, a specific block in the process of transduction which did not affect recombination if the donor DNA was introduced by conjugation. This strain also had a much lower reversion rate to histidine or proline independence than its parent (Stacey & Lloyd, 1976). The mutant has, therefore, been re-examined in detail and the results are reported in this paper. The observations made by Lloyd have been confirmed and extended and, in addition, it has been shown that one mutation was responsible for four changes in the phenotype: it could not be transduced; transducing phage could not be obtained from it; it could only rarely be transformed by R factor DNA; and it had an abnormally low rate of mutation.

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METHODS

Bacterial and bacteriophage strains. The strains of *E. coli* K12 and the bacteriophage used in this investigation are listed in Table 1. The locations of relevant genetic markers can be found in Taylor & Trotter (1972) and, for F' factors, in Low (1972).

Chemicals. All amino acids, vitamins, enzymes and antibiotics were from Sigma, except ampicillin, which was bought under the trade name of Penbritin from Beecham Research Laboratories. Other reagents were from Fisons Scientific Apparatus, and were of analar grade where obtainable.

Media. Nutrient broth, nutrient agar and tryptone broth were made as described by Evenchik, Stacey & Hayes (1969). Minimal agar was Davis salts (Davis & Mingioli, 1950) solidified with Davis New Zealand agar (15 g l^{-1}) and supplemented with the appropriate sterile amino acids at a final concentration of $20 \mu\text{g ml}^{-1}$, carbon source at 0.4% (w/v), vitamins at $1 \mu\text{g ml}^{-1}$ and antibiotics at $20 \mu\text{g ml}^{-1}$. Liquid minimal medium was M9 salts as described by Anderson (1946). The medium for transformation experiments was the P medium of Kaiser (1962) supplemented with $100 \mu\text{g ml}^{-1}$ of the required amino acids. The dilution fluid was phage buffer (Clowes & Hayes, 1968) except when handling bacteriophages P1 and Mu. P1 adsorption fluid was 10% (v/v) tryptone broth with 0.01 M- CaCl_2 . This was supplemented with 0.005 M- CdSO_4 for bacteriophage Mu. P1 saline contained 1 mM- MgSO_4 , 0.1 mM- CaCl_2 and 0.001% (w/v) gelatine in isotonic saline solution. Dorset egg slopes were 75% (v/v) beaten egg, 25% (v/v) nutrient broth with 0.1% (w/v) thymine, solidified by heating to 80°C for 1 h.

Growth of strains. Bacterial stock cultures were maintained on Dorset egg slopes from which nutrient agar plate cultures were made at weekly intervals. Single colonies were inoculated into 5 ml nutrient broth and incubated overnight at the appropriate temperature. Experimental cultures were obtained by a 1:50 dilution into warm fresh nutrient broth and incubated on a Luckhams shaker until the exponential phase of growth.

Bacteriophage cultures were stored at 4°C in the appropriate dilution fluid. Lysates were prepared by the confluent lysis plate method (Adams, 1959).

Transduction experiments. The method of Lennox (1955) was used for bacteriophage P1 transductions with the substitution of P1 adsorption fluid for saline. When selecting for drug resistance, the bacteria were incubated for 1 h in nutrient broth to permit expression of the drug-resistance genes of the plasmid before plating on selective media. Transductions with bacteriophage Mu were performed by the method of Howe (1973) using the appropriate diluent. The transduction frequencies referred to in the text are expressed in terms of the number of transductants per input phage.

For ultraviolet (u.v.) irradiation of transducing particles, bacteriophage lysates were spun for 90 min at $18000 \text{ rev. min}^{-1}$ on an MSE18 centrifuge, the supernatant was discarded and the phage pellet was gently resuspended in P1 saline. The suspension was then irradiated using a Hanovia UV lamp at a distance of 59 cm and a dose rate of approximately $1 \text{ J m}^{-2} \text{ s}^{-1}$. Lysates showing a 1% survival were used.

Transformation. The plasmid DNA was prepared by the lysozyme-SDS method followed by dye-buoyant density centrifugation (Radloff, Bauer & Vinograd, 1967). Exponentially growing cultures of MRW24(BN21) in P medium were harvested by centrifugation, washed and resuspended in 25 ml TES buffer (0.02 M- NaCl /0.02 M- Tris /1 mM-EDTA, pH 8.0). Lysozyme was added to a final concentration of 1 mg ml^{-1} and the suspension was incubated at 37°C for 1 h. SDS was then added to a final concentration of 1% (w/v) and the incubation was continued for a further 10 min. Finally, self-digested RNAase was added ($500 \mu\text{g ml}^{-1}$)

Table 1. *Bacteria, plasmids and bacteriophages*

	Relevant genotype	Source or derivation
<i>E. coli</i> K12 strain		
S49I	<i>pro his lac gal</i> AzaU ^R λ^- λ^S F ⁻ <i>sup</i> ⁺	Stacey & Lloyd (1976)
R272I	as S49I, but <i>tdi</i>	Lloyd (1971)
S49I λ^R	as S49I, but λ^R	From challenge with λ_V
R272I λ^R	as R272I, but λ^R	From challenge with λ_V
S49I(P1)	as S49I, but lysogenic for phage P1	This work
R272I(P1)	as R272I, but lysogenic for phage P1	This work
AB1157	<i>thr leu his pro arg thi gal lac ara mtl</i> F ⁻ λ^- λ^S Sm ^R	P. Howard-Flanders (see Bachmann, 1972)
W4520	<i>gal</i> ₄ / <i>Fgal</i>	P. Howard-Flanders
C600	<i>thr leu thi lac</i> F ⁻ Sm ^R	Laboratory stock strain
R9	as AB1157 <i>tdi leu</i> ⁺	This work
4036	(λ) (λ dgal) <i>gal sup</i> ⁺	Laboratory stock strain (w3350)
E126	<i>leu nadC</i>	J. R. Guest
MRW24	HfrH <i>thi su endo</i> ₁ <i>bio</i>	K. Buxton
Plasmid		
<i>Fgal</i>	<i>gal</i> ⁺	—
KLF1		B. Low
KLF4		B. Low
R1	Cm ^R Sm ^R Su ^R Pc ^R Km ^R	J. Holton
BN21	as R1, but Km ^S <i>tra</i>	J. Holton
R237	Cm ^R	J. Holton
R124	Tc ^R	J. Holton
ColE ₂		J. Holton
F101		J. R. Guest
Bacteriophage		
λ	Wild type	Laboratory stock
λ c1	Clear plaque mutant	Laboratory stock
P1 kc	Clear plaque mutant	Laboratory stock
Mu-1		P. van de Putte
P1CM	Cm ^R	J. L. Rosner
MS2		J. Holton
T6	Wild type	Laboratory strain

Abbreviations used throughout this paper are: AzaU, azauracil; Nal, nalidixic acid; Val, valine.

and the incubation was continued for a further 30 min. The crude lysate (1 ml) was layered on to the top of 3 ml saturated CsCl solution in a 5 ml polycarbonate centrifuge tube, 1 ml ethidium bromide solution (4 mg ml⁻¹) was added and the tube was inverted to mix the solutions. The mixture was spun, using a 3 × 5 ml rotor in an MSE50 centrifuge, at 45000 rev. min⁻¹ for 40 h. The band of plasmid DNA, located using a u.v. lamp, was removed by puncturing the tube just below the band with a syringe and withdrawing 0.2 ml. The ethidium bromide was removed by mixing with an equal volume of isopropyl alcohol and discarding the (upper) alcoholic layer containing the dye. The remaining traces of alcohol were removed by overnight dialysis against TES buffer. DNA concentrations were determined by the method of Burton (1956).

For the transformation experiments, the techniques for the growth and preparation of the recipient bacteria and the transformation procedure itself were those described by Cohen, Chang & Hsu (1972). Samples were plated on the appropriate selective media after incubation for 1 h in growth media to permit expression of the drug-resistance phenotype. The frequency of transformation was determined from viable counts at the end of the transformation period.

Mutation studies. Cultures were grown to the exponential phase in nutrient broth, harvested by centrifugation, washed with warm phage buffer and resuspended in 0.1 vol. warm phage buffer. Samples were then plated out on the appropriate media and viable counts were determined in the usual manner. For u.v. mutagenesis, bacteria suspended in phage buffer were diluted to 10^7 ml⁻¹ and irradiated under conditions identical to those used for irradiating phage suspensions. Samples were concentrated by centrifugation before plating out.

Mating experiments. Conjugations were performed using exponential-phase cultures, with a ratio of male:female bacteria of 1:10. In mapping experiments, the mating culture was diluted 100-fold into warm nutrient broth 5 min after mixing. Samples were plated on the appropriate selective media. 6-Azauracil (100 µg ml⁻¹) was used to counterselect the male bacteria. The nutritional phenotype of the recombinants was determined by replica-plating (Lederberg & Lederberg, 1962). The restriction/modification phenotype of the recombinants was determined by measuring the plating efficiency of λ CI.K12 and λ CI.C.

Chromosome mobilization by an F' factor. An *Fgal* factor was introduced into s491 and R2721 by mating with W4520. These new donor strains were then used in matings with AB1157 and C600 selecting for transfer of the *Fgal* and also for the chromosomal markers, *thr*⁺ and *leu*⁺.

Frequency of lysogenization by P1CM. The method of Rosner (1972) was used.

RESULTS

Failure of transduction in R2721

Lloyd (1971) found no Pro⁺ transductants after P1 infection of R2721. This result was confirmed by attempts to transduce histidine or proline independence using lysates of phage P1 grown on several different hosts. The absence of transductants was made the more striking by the virtual absence of spontaneous revertants. Although prior u.v. irradiation of P1 lysates increases the yield of transductants in normal strains by a factor of 3 to 5 (Arber, 1960; Wilson, 1960) and in *recB* mutants by a factor of 50 (Akman, Atkinson & Stacey, unpublished), there was no effect on the yield in R2721 although it stimulated the yield of transductants in the parental strain, s491, more than is normally observed. A P1 lysate which gave 12.5 times as many His⁺ transductants with s491 after irradiation to a phage survival of 4.2% still gave no transductants with R2721.

In order to demonstrate that transducing DNA could enter R2721, the transduction of a complete replicon, in this instance an R factor, had been attempted by Lloyd (1971) and it had failed. This failure was disconcerting: if the trivial explanation, a failure of transducing DNA to enter the cell, could be ruled out, it implied the necessity for a recombination step (or other host-mediated process) in the transduction of an episome. Because R2721, as will be shown below, was a reasonable host for phage P1 we repeated this experiment with different R factors (R1, R237, R124), but with the same result. With the R factor BN21, a transfer-deficient derivative of R1, the deficiency in transduction of R2721 was even more striking because the yield in s491 was better even than that obtained for single chromosomal markers (Table 2).

An increase in the multiplicity of infection did not produce transductants in R2721, implying that P1 itself could not assist in transduction. Moreover, P1 lysogens of R2721 were no different from non-lysogenic derivatives. However, transductants were obtained with R2721 as recipient if the phage was grown on 4036, which contains λ dgal. Selection on galactose-minimal agar gave Gal⁺ transductants in λ -resistant derivatives of both mutant

Table 2. P1 transduction of plasmids

Transductants were tested for cotransduction of other resistance markers by replica-plating. In the case of transduction of *Fgal*, *Gal*⁺ colonies were tested for 'maleness' with the sex specific phage MS2.

Plasmid	Selected marker	Frequency of transduction per adsorbed phage	
		S491	R2721
BN21	Cm ^R	1.5×10^{-5}	ND*
R124	Tc ^R	1.8×10^{-7}	ND
R237	Cm ^R	3.2×10^{-6}	ND
<i>Fgal</i>	<i>gal</i> ⁺	4.4×10^{-7}	ND

* ND, None detected.

and parental strains: the yield for R2721 λ^R (1.2×10^{-6}) was nearly the same as that for S491 λ^R (4.2×10^{-6}).

Bacteriophage Mu also acts as a vector for generalized transduction (Howe, 1973) with a yield of transductants one tenth that mediated by P1. Although less easy to demonstrate, the difference between R2721 and S491 in the yield of Mu-mediated transductants appeared to be the same as for P1: none were detected for R2721 while the frequency for the *pro*⁺ marker in S491 transductants was 2.3×10^{-7} .

P1 infectivity and growth in R2721

The failure to obtain P1 transductants in R2721 could have arisen from trivial differences in the processes of adsorption and infection. P1 adsorption was shown to be only slightly lower (90%) than that found for S491, under the conditions used for transduction, but the efficiency of plating was always less on lawns of R2721 as host. Another indication that P1 DNA was injected into R2721 was obtained from experiments measuring the frequency of lysogenization with P1CM, a derivative of P1 which carries the gene for resistance to chloramphenicol derived from an R factor (Kondo & Mitsuhashi, 1964). The frequencies of lysogenization for S491 and R2721 were similar (18% for S491 and 22% for R2721 when the multiplicity of infection was 2).

Transformation

Escherichia coli is not transformable under normal cultural conditions. However, after extensive treatment in CaCl₂ solutions, transformants have been obtained with exogenous DNA (Mandel & Higa, 1970; Cohen *et al.*, 1972). Most of the linear DNA which enters the cell in this way is degraded to acid-soluble products by the action of the *recBC* gene product, exonuclease V (Wackernagel, 1973). Thus, only covalently closed circular DNA can successfully transform wild-type cells. As the mutant R2721 was defective in the inheritance of a plasmid which had been introduced by transduction, it was decided to test whether transformation with a plasmid was possible.

Although it was possible to transform the parental strain normally, R2721 was transformed only at a very low frequency; at the same concentrations of DNA, S491 gave 10000 times more transformants per ml than R2721.

Mutation rate of R2721

The frequency of revertants to independence of proline, 4.4×10^{-7} , and of histidine, 2.2×10^{-7} , found in this study agree closely with those reported by Lloyd (1971) for S491. The frequency of revertants for these two markers in cultures of R2721 were at least two orders of magnitude lower, approximately 1 in 10^9 cells plated. It was difficult to establish

Table 3. *Mutation rates*

Mutant phenotype	Frequency of mutants			
	s491	R2721	C600	R9†
Pro ⁺	4.4×10^{-7}	ND*	—	—
His ⁺	2.2×10^{-7}	ND	—	—
Nal ^R	1.7×10^{-7}	$< 10^{-9}$	3.1×10^{-7}	$< 10^{-9}$
Val ^R	3.2×10^{-8}	$< 10^{-7}$	—	$< 10^{-9}$
T6 ^R	2.6×10^{-6}	3.2×10^{-6}	—	—
ColE ₂ ^R	1.6×10^{-6}	1.2×10^{-6}	—	—

* ND, None detected under conditions when 10^8 to 10^9 bacteria were plated on selective plates.

† R9 is a recombinant isolated from a cross, R2721(*Fgal*) × ABI157. It appeared Tdi⁻ because of its low mutation rate, and its inability to give transductants after P1 infection for either chromosomal or plasmid markers. It had not inherited the slow growth marker of R2721 and was more resistant to u.v. light than ABI157.

the true reversion rate because most of the revertants for one marker had regained the ability to revert normally at the other locus and to be transduced for chromosomal and plasmid markers. It was this finding that suggested that a single mutation was responsible for both these characteristics. All of 20 'revertants' tested had regained the Tdi⁺ phenotype when these rare colonies were picked. Indeed the presence of a significant number of revertants in a culture of R2721 was regarded as evidence that it contained revertants of the *tdi* mutation and it was discarded.

Under normal circumstances u.v. irradiation increases the mutation rate, especially for amber and ochre suppressor mutations, but it had no effect on the yield of revertants with R2721. At 1% survival, the frequency of His⁺ cells is increased 10-fold in s491. Although the mutant also had very low rates of mutation to drug resistance, the yields of mutants resistant to bacteriophages and colicins were not greatly different from those obtained with s491 (Table 3).

Chromosome mobilization

An *Fgal* was introduced into s491 and R2721 by mating with W4520 and these plasmid-bearing derivatives were used as donors in mating experiments with ABI157 and C600 as recipients; Gal⁺ and Thr⁺ Leu⁺ recombinants were selected on the appropriate minimal media. The frequency of mobilization was slightly higher with R2721(*Fgal*) than with s491(*Fgal*) implying that there was no defect in the recombination between the episome and the chromosome. In a typical experiment in which the yield of ABI157(*Fgal*) was the same for both donors, the chromosome mobilization index for s491(*Fgal*) was 2.4×10^{-3} and for R2721(*Fgal*) it was 5.3×10^{-3} .

Absence of transducing particles in P1 lysates grown on R2721

High-titre stocks of bacteriophage P1 grown on strain R2721 were used to attempt the transduction of ABI157 and C600 (Table 4). No transductants were obtained even though the same markers were transferred to these strains by R2721(*Fgal*). The transduction of R1 from R2721(R1) was similarly unsuccessful. These findings implied that mapping the *tdi* mutation by transduction would be impossible.

Mapping the tdi mutation

The mutant strain, R2721, showed, in addition to the phenotypic characters already described, two other differences from its parental strain: it was more u.v.-sensitive and its growth was much slower, even on rich media. It was necessary to establish whether these

Table 4. *Transduction with P1.R2721*

Donor	Recipient	Transductants per phage adsorbed			
		Thr ⁺	Leu ⁺	Arg ⁺	R1
S491(R1)	AB1157	3×10^{-6}	1.5×10^{-6}	4×10^{-6}	3×10^{-5}
R2721(R1)	AB1157	ND*	ND	ND	ND
R9†	AB1157	ND	—	ND	—

* ND, None detected.

† R9 is a Tdi⁻ recombinant of AB1157.

Table 5. *Analysis of recombinants between HfrH and R2721*

100 Pro⁺ recombinants were picked, purified and tested for the unselected markers.

Selected marker <i>pro</i> ⁺	Unselected markers (%)		
	<i>tdi</i> ⁺⁺	UV ^R †	Large colony‡
	76	86	90

* The efficiency of transduction of chloramphenicol resistance from C600(BN21) and the reversion rate for histidine independence were measured to assess this property.

† The approximate survival curves were obtained by spotting a range of dilutions on plates and exposing them to given doses of u.v. radiation.

‡ The sizes of single colonies were compared after incubation overnight on nutrient agar plates. The distinction between the small colony size of R2721 and the normal colony size was quite unambiguous.

Table 6. *Analysis of recombinants between R2721(Fgal) and E126*

150 Leu⁺ recombinants from a single cross were picked and purified on the same selective medium and tested for the unselected markers.

Selected marker <i>leu</i> ⁺	Unselected donor markers (%)			
	<i>nadC</i> ⁺	<i>tonA</i>	<i>tdi</i> [*]	<i>pro</i>
	88	72	62	42

* The Tdi character was determined by measuring the efficiency of transduction, by P1 grown on C600(BN21), of resistance to chloramphenicol, and the reversion rate to proline independence of *pro* clones was also checked. In all cases the absence of transduction and reversion were found to occur together.

Table 7. *Analysis of recombinants between E126(F101) and R2721*

150 Pro⁺ recombinants from a single cross were picked, purified and tested for the unselected markers.

Selected marker <i>pro</i> ⁺	Unselected donor markers (%)		
	<i>nadC</i>	<i>tonA</i> ⁺	<i>tdi</i> ⁺⁺
	39	53	80

* The Tdi phenotype was established by measuring the frequency of chloramphenicol-resistant transductants following infection with P1 grown on C600(BN21) and by measuring the reversion rate to histidine independence.

changes were associated with the *tdi* mutation or if other mutations, induced at the original mutagenesis, were responsible by transfer of the new mutation(s) into different genetic backgrounds. Revertants of R2721 to *tdi*⁺ remained slow growing and u.v.-sensitive while occasional large colony forms remained *tdi*.

The chromosome mobilization experiments generated recombinants in two different

genetic backgrounds, C600 and AB1157, which could be tested for the R2721 phenotype. These showed that two other mutations were responsible for the slow growth and the u.v. sensitivity and that all three mutations appear to lie in the segment of the chromosome between *thr* and *pro*. The nature of the other two were not investigated further. These results were confirmed by a cross between HfrH and R2721 (Table 5).

To locate the *tdi* mutation more precisely, experiments were performed using a *tonA*⁺ *nadC* recipient strain, E126, the gift of Dr J. R. Guest. The results (Tables 6 and 7) show that the *tdi* mutation lies between *tonA* and *pro*. The wild-type allele, *tdi*⁺, is carried by the episome KLF4 but not by KLF1 or *Fpro lac*. Strains heterozygous for this locus have wild-type characteristics; the *tdi* mutation is recessive. Only strains carrying KLF4 reverted to independence of histidine at the wild-type rate and had gained the ability to be transduced by P1 to chloramphenicol resistance.

These experiments allowed us to check the effects of the *tdi* mutation in another genetic background and, as far as they were tested, the features of the phenotype were the same in C600 Tdi⁻ recombinants. The absence of transduction was not modified at all by transfer to a strain that is a good host for phage P1 nor were there any transducing particles in a P1 lysate grown on R9 (Table 4).

DISCUSSION

A single mutation, in a hitherto unknown gene to be given the non-committal symbol *tdi*, appears to be responsible for a number of related changes in the phenotype of R2721. This strain could neither be transduced by bacteriophage P1 nor give rise to transducing particles: it could only rarely be transformed by R factor DNA and exhibited a very low mutation rate. The failure to be transduced by P1 is unlikely to be the result of poor adsorption of the bacteriophage as the mutant was a reasonable host for this phage: it gave high-titre lysates of P1 and was readily lysogenized by P1CM. Moreover, a similar defect was observed when bacteriophage Mu was used as the vector. The best proof that transducing DNA could enter the cell intact was the demonstration that Gal⁺ transductants could be obtained when the donor strain carried the defective phage λ gal. In this case, presumably, the block is circumvented by the λ -coded enzyme, integrase (Gottesman & Weisberg, 1971).

While it is true that restriction has a greater effect on the yield of transduction than on the yield of recombinants following conjugation, it would be difficult to explain the striking difference in the yields of recombinants by a change in the host specificity of R2721. There was no evidence that it differed from S491 as far as tests with phage λ would reveal; the efficiencies of plating of λ grown on standard strains of *E. coli* K12, B and C were the same on both strains and there was no difference between λ .S491 and λ .R2721 in their ability to form plaques on any of the strains tested. More convincing was the observation that in the recombinants obtained in crosses with R2721(*Fgal*) as donor and C600 r⁻m⁻ as recipient, the *hsd* specificity markers segregated quite independently of *tdi*.

The failure to observe any enhancement of the mutation rate of R2721 by u.v. irradiation implies that repair replication occurs exclusively by error-free mechanisms, a phenotype similar to that associated with the *lex* mutation. In *lex* mutants, however, there is a high level of recombination by transduction, only slightly less than that in the wild type (Witkin, 1969*a, b*; Stacey, unpublished observations).

The observation that transduction and transformation failed for R factors suggests that the establishment of these supernumerary chromosomes requires a recombinational event. This is not surprising for transduction because the circular chromosome is broken during the transfer process but in the transformation experiments only covalently closed circles of

plasmid DNA were used. In the case of viruses like P1, circularization is thought to occur by recombination between the regions of terminal redundancy present in the vegetative viral DNA (Ikeda & Tomizawa, 1965).

We have no other experimental data that bears on the nature of the defect in this mutant. Labelled transducing DNA is not rapidly degraded; it persists in a high molecular weight form within the cell for at least 60 min. This again suggests that the block in its incorporation is not due to restriction. Moreover double mutants with the *recA* and *recB* mutations were viable and their phenotypes had no unusual features.

The most striking difference between transducing DNA and DNA at the time of entry during conjugation is that one is thought to be double-stranded and the other single-stranded (Siddiqi & Fox, 1973; Ebel-Tsipsis, Botstein & Fox, 1972). It is believed that transforming DNA is reduced to a single strand on entry in certain bacterial species (Dubnau & Cirigliano, 1972). It is tempting to suggest that an additional mechanism is required to bring transducing DNA into a recombinational pathway, the *tdi* mutation defining this step. However, the absence of any effect on recombination of u.v. irradiation of the transducing particles and the rarity of mutation suggests that the defect is at a later stage in recombination.

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