Generalized Transduction between *Salmonella typhi* and *Salmonella typhimurium* by Phage j2 and Characterization of the j2 Plasmid in *Escherichia coli*

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Phage j2, a P1-like phage in *Salmonella typhi*, was heteroimmune to phage P1 and existed in the lysogenic state as a plasmid of molecular size 58.6 MDal. The phage j2 plasmid was incompatible with the P1 plasmid (IncY group). A j2-sensitive mutant of *Salmonella typhimurium* LT2 was isolated by transduction of j2Ap phage into LT2 followed by curing of the prophage. The mutant was used to demonstrate transduction between *S. typhi* and *S. typhimurium* by phage j2.

INTRODUCTION

Mise *et al.* (1981) showed that phage j2 of *Salmonella typhi* (Anderson, 1959; Bernstein & Wilson, 1963) was a generalized transducing phage similar to P1 of *Escherichia coli*. Although j2 formed plaques on all *S. typhi* strains tested, it did not do so on those of *E. coli* K12, B, C, or *Salmonella typhimurium* LT2 (Mise *et al.*, 1981). There were two objectives in the present study: further characterization of phage j2 in comparison with phage P1, and the establishment of j2-mediated transduction between *S. typhi* and *S. typhimurium*.

METHODS

Bacteria, phages and plasmids. The organisms and plasmids used are listed in Table 1.

Media, assay of phage and transduction. These were described by Mise *et al.* (1981).

Tests for incompatibility. Those of Chabbert *et al.* (1972) were used.

Isolation of j2Ap and D6Cm. These P1-R hybrid phages were obtained by the enrichment technique of Mise & Arber (1976).

(i) Isolation of j2Ap. *Salmonella typhi* strain MK1005 (j2 + R1) was UV-irradiated in order to obtain a j2 phage lysate, and 10^8 p.f.u. of the lysate were mixed with 5×10^8 cells of strain A. After 15 min adsorption at 37 °C, the infected cells were sedimented and the pellet spread on five plates of Bacto-Penassay broth (PAB; Difco) agar containing ampicillin at 25 µg ml⁻¹. The frequency of transduction of the ampicillin resistance (Ap) marker was 2×10^{-5} per p.f.u. All Ap^r transductants on the plates were pooled in fresh L broth (Lennox, 1955), diluted to 10^8 cells ml⁻¹, and incubated for 90 min. A phage lysate was again obtained by UV-induction from the Ap^r transductants. The lysate transduced the Ap marker to strain A at a higher frequency (5×10^{-3} per p.f.u.). About 300 Ap^r transductants were pooled, diluted, incubated in L broth and UV-irradiated to induce j2 phages. The resulting phage lysate transduced the Ap marker to strain A at a yet higher frequency (2×10^{-1} per p.f.u.). All 24 Ap^r transductants were lysogenic for phage j2Ap.

(ii) Isolation of D6Cm. Phage D6Cm was obtained by essentially the same method as described above except that (a) *Escherichia coli* strains WA921(D6 + NR1) and WA921 were used instead of MK1005(j2 + R1) and A; and (b) PAB agar supplemented with chloramphenicol at 25 µg ml⁻¹ was used instead of ampicillin-containing agar.

Abbreviation: PAB, Penassay broth.

Table 1. *Organisms and plasmids used*

	Properties	Source or reference
Bacteria:		
<i>Salmonella typhi</i>		
j2	j2 lysogen	Anderson (1959)
A	<i>trp met</i>	Craigie & Yen (1938)
MK1001	<i>trp met phe</i>	} derivatives of A Mise <i>et al.</i> (1981)
MK1002	<i>trp met arg</i>	
MK1003	<i>trp met thr</i>	
MK1004	<i>trp met pro</i>	
MK1005	<i>trp met Rif^R Nal^R</i>	
MK1006	<i>trp met his</i>	
MK1008	<i>trp met xyl</i>	
MK1009	<i>trp met mal</i>	
<i>Salmonella typhimurium</i> LT2		
CC4415	<i>trp met val</i>	Mise (1976)
MK1046	j2 sensitive	CC4415
<i>Escherichia coli</i> C		
wild type	restriction negative	
<i>Escherichia coli</i> K12		
WA921	<i>thr met leu hsdK</i>	Wood (1966)
Bacteriophages:		
j2		Anderson (1959)
j2Ap		this paper
P1		Lennox (1955)
P1Cm1		Mise & Arber (1976)
P1Tc1		Mise & Arber (1976)
D6		Mise & Suzuki (1970)
D6Cm		this paper
P22		Zinder & Lederberg (1952)
Plasmids:		
S-a	CmSmSuKm	Watanabe <i>et al.</i> (1968)
NR1	CmSmSuTcHg	Rownd <i>et al.</i> (1966)
NR1-RTF	Tc	Mise <i>et al.</i> (1981)
R1	ApCmSmSu	Lawn <i>et al.</i> (1967)

Both D6Cm and j2Ap could multiply without the help of the wild-type phage. The transduction frequency of the resistance marker by both phages was 1.0 per p.f.u.

Isolation of plasmid DNA. The method of Kado & Liu (1981) was used.

Agarose gel electrophoresis of plasmid DNA. This was as described by Tanaka *et al.* (1976); 0.7% agarose was used.

RESULTS AND DISCUSSION

Characterization of j2Ap phage

Biochemical characterization of the j2 phage and plasmid was mainly carried out in *S. typhimurium* or *E. coli*, since *S. typhi*, its original host, is pathogenic. Phage j2Ap was very similar to phage j2; for example both formed tiny plaques and required Ca^{2+} for adsorption. Unlike phages P1Cm (Kondo & Mitsuhashi, 1964) and λ Cm (MacHattie & Jackowski, 1977) during propagation less than 1% of j2Ap progeny lost the Ap marker as determined by a plaque-centre test (Kondo & Mitsuhashi, 1964). The frequency of transduction of the Ap marker by j2Ap to *S. typhimurium* or to *E. coli* was much lower than that to *S. typhi* (Table 2), which is not surprising since neither *E. coli* or *S. typhimurium* is sensitive to j2. More than 90% of the Ap^r transductants of *E. coli* or *S. typhimurium* strains were lysogenic for j2Ap and upon UV irradiation produced a lysate of $> 10^6$ p.f.u. ml⁻¹. This suggested that the Ap marker was transduced to these strains by lysogenization with j2Ap. Non-lysogenic derivatives of *E. coli* C or *S. typhimurium* CC4415 were isolated from lysogens by UV irradiation and subsequent incubation in L broth. Ninety-two percent of those from *S. typhimurium* were j2-sensitive, but none from *E. coli* was sensitive to j2

Table 2. Transduction of the *Ap* marker by phage j2Ap (grown on *S. typhi* A)

Recipient	Frequency of transduction (per p.f.u.)*
<i>S. typhi</i> A	1.0
<i>E. coli</i> C	$3 \pm 2 \times 10^{-7}$
<i>S. typhimurium</i> CC4415	$3 \pm 1 \times 10^{-3}$
<i>E. coli</i> C 'j2Ap cured'	$3 \pm 2 \times 10^{-7}$
<i>S. typhimurium</i> CC4415 'j2Ap cured'	1.0

* The values are means of three independent determinations.

Table 3. Transduction between *S. typhimurium* and *S. typhi* by phage j2

Donor	Recipient	Selected marker	Transduction frequency (per p.f.u.)*
MK1046	MK1001	<i>phe</i> ⁺	4×10^{-7}
MK1046	MK1002	<i>arg</i> ⁺	2×10^{-7}
MK1046	MK1003	<i>thr</i> ⁺	8×10^{-8}
MK1046	MK1004	<i>pro</i> ⁺	1×10^{-6}
MK1046	MK1006	<i>his</i> ⁺	2×10^{-7}
MK1046	MK1008	<i>xyl</i> ⁺	2×10^{-8}
MK1046	MK1009	<i>mal</i> ⁺	2×10^{-7}
MK1005(NR1)	MK1046	Tc	6×10^{-6}
MK1005(S-a)	MK1046	Cm	2×10^{-6}

* The values are means of two independent determinations.

in spite of five independent trials. A j2 lysate (10^8 p.f.u. ml⁻¹) was obtained by growth on a j2-sensitive derivative of CC4415, designated MK1046. Plaques of j2 were bigger on MK1046 than on *S. typhi* A.

Heteroimmunity of j2 and P1

Phage j2 did not form plaques on *S. typhi* lysogenic for P1, as a result of poor adsorption of j2 to the P1 lysogen (Mise *et al.*, 1981), suggesting that P1 might be a converting phage which induces the production of new surface components. To investigate the immunity relationship of these two phages, the plaque-forming ability of P1 on *E. coli* C(j2Ap) was compared with that on *E. coli* C. The P1 plaque-forming efficiency on *E. coli* C(j2Ap) was 0.8 ± 0.1 per p.f.u. on *E. coli* C (means of three independent determinations), clearly indicating that phages j2 and P1 are heteroimmune.

Generalized transduction between *S. typhi* and *S. typhimurium*

Isolation of j2-sensitive derivatives of *S. typhimurium* permitted tests of transduction between *S. typhi* and *S. typhimurium* (Table 3). Phage j2 transduced all the seven chromosomal genetic markers tested, and both plasmid markers, from *S. typhimurium* to *S. typhi*. Frequencies of transduction of the chromosomal markers ranged from 1×10^{-6} to 2×10^{-8} per p.f.u., values which are 10 to 130-fold less than between strains of *S. typhi* (Mise *et al.*, 1981); this may reflect differences between these two salmonellas in chromosomal DNA base sequences. P1-sensitive mutants of many Gram-negative bacteria could be isolated by transduction of the kanamycin resistance (Km) marker of P1Km phage and subsequent curing of the P1Km prophage (Goldberg *et al.*, 1974). These authors were unable to isolate P1-sensitive mutants of *S. typhimurium*, probably because *Salmonella* is a poor host for P1 either as a lysogen or lytically. Although unstable (Mise *et al.*, 1981), with a half-life of 3.5 d at 4 °C, phage j2 could be useful in answering this problem and act as a P1-like generalized transducing phage for *S. typhimurium* as well as *S. typhi*.

Fig. 1. Comparison of plasmid size by agarose gel electrophoresis. Slots: A, NR1-RTF; B, P1Tc; C, P1; D, j2; E, j2Ap; F and G, hybrids between j2Ap and P1Tc. Host strains of these plasmids were WA921 for NR1-RTF, P1Tc and P1, C for j2Ap and the hybrids, and A for j2, respectively. The molecular masses of the plasmids and transposons used to calibrate the gels are: 49.0 MDal for NR1-RTF (Perlman & Rownd, 1976); 58.6 MDal for P1 (Arber *et al.*, 1978); 3.2 MDal for Tn3 (Ohtsubo *et al.*, 1978); and 6.0 MDal for Tn10 (Tye *et al.*, 1974).

Table 4. *Incompatibility of j2 and P1 plasmids*

E. coli C lysogenic for P1 group phages was superinfected with P1Tc or D6Cm at a multiplicity of infection of $<10^{-2}$. The infected cells were plated on medium containing either tetracycline or chloramphenicol. Resistance patterns of the superinfected cells were tested after one subculture on the same medium.

Superinfecting phage	Recipient lysogen	Selected marker	Transduction frequency of resistance marker (per p.f.u.)	Percentage of transductants carrying two resistance markers
P1Tc	C(j2Ap)	Tc	2×10^{-1}	53 (TcAp)
D6Cm	C(j2Ap)	Cm	3×10^{-1}	100 (CmAp)
P1Tc	C(P1Cm)	Tc	5×10^{-1}	0 (CmTc)

Incompatibility of prophage j2 and P1 plasmids

Prophage P1 is an extrachromosomal replication unit (plasmid) in *E. coli* lysogenic for P1 (Ikeda & Tomizawa, 1968). To test whether j2 prophage was similar to P1, cleared lysates of j2 lysogens were subjected to agarose gel electrophoresis. Covalently closed circular DNA occurred in j2 lysogens, but not in non-lysogens, suggesting that the j2 prophage exists as a plasmid. The j2 plasmid DNA was almost the same in size as the P1 DNA (58.6 MDal: slots C and D in Fig. 1 and Arber *et al.*, 1978). P1 and j2 plasmid DNAs were not separated in agarose gels when both the DNAs were mixed, confirming that the molecular weight of the j2 plasmid is very close to that of P1 (data not shown). Although the difference in size between j2 and j2Ap DNA was slight (slots D and E in Fig. 1), it was significant and reproducible in five independent experiments. The result suggests that j2Ap phage might have been formed by transposition of Tn3 from R1 to the j2 genome.

P1 and P7 plasmids belong to the same incompatibility group, IncY, whereas D6 belongs to an unidentified but different incompatibility group (Watkins & Scott, 1981). To determine whether j2 belonged to the P1-P7 or D6 incompatibility group, *E. coli* C(j2Ap) was superinfected with P1Tc or D6Cm and cells with the antibiotic resistance marker of the superinfecting phage were selected. After one subculture on the same selective medium, a single colony of each clone was tested for the Ap marker of j2Ap (Table 4). The j2 plasmid was excluded from *E. coli* C by the

incoming P1 plasmid but not by D6. P1-mediated exclusion of P1 was very strong as compared to that of P1 by j2.

Forty-two of the 50 Ap^rTc^r derivatives of *E. coli* C obtained by superinfection of C(j2Ap) with P1Tc were unstable, and lost one of the resistance markers at high frequencies (more than 10%), when grown in PAB containing neither ampicillin nor tetracycline at 37 °C overnight. Generally, cultures of the Ap^rTc^r strains contained a large number of phage particles; when 3×10^7 cells ml⁻¹ of an Ap^rTc^r strain were incubated in PAB without antibiotics at 37 °C for 5 h with aeration, the culture contained 7×10^7 p.f.u. ml⁻¹ on *E. coli* C and 5×10^4 p.f.u. ml⁻¹ on *S. typhi* A. Apparently, the Ap^rTc^r strain temporarily carried both j2Ap and P1Tc genomes in a cell in an unstable state and released the phages during growth. Hybrid P1 and j2 phages which could grow on both *S. typhi* A and *E. coli* C were not found in the culture. Stable Ap^rTc^r strains were obtained by superinfection of C(j2Ap) with P1Tc, following isolation on selective medium containing ampicillin and tetracycline and by repeated subculture of the doubly resistant clones on the same medium. Twenty-two of 24 stable Ap^rTc^r strains produced plaque-formers on *E. coli* C alone upon UV irradiation. These Ap^rTc^r strains contained a plasmid whose molecular weight value was slightly greater than that of P1Tc (slots F and G in Fig. 1). The plasmid might have resulted from transposition of Tn3 to the P1 genome or recombination between P1Tc and j2Ap under selective pressure.

The above results indicate that j2 and P1 plasmids are incompatible, while j2 and D6 are compatible. Phage P1 was isolated from *E. coli* and D6 from *Salmonella oranienburg* (Bertani, 1951; Mise & Suzuki, 1970). It is interesting that phage j2 isolated from *Salmonella typhi* belonged to the P1 but not the D6 incompatibility group.

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