119. Lysogenic Conversion and Transduction of Genetic Characters by Temperate Phage Iota in *Salmonella*.

II. Transduction of Genetic Characters by Phage Iota in *Salmonella* Groups A, B, and D

By Shoei ISEKI and Kanichi KASHIWAGI

Department of Legal Medicine, School of Medicine,
Gunma University, Maebashi, Japan

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Zinder and Lederberg¹⁾ found out that various genetic characters, for examples, nutritional factor, fermentation of carbohydrate, and streptomycin resistance were transduced by temperate phage from one strain to another of *Salmonella typhi murium* and others of *Salmonella* groups B and D. This phenomenon, which is called transduction, was later confirmed by many workers. Recently, Demerec, Blomstrand, and Demerec²⁾ used this phenomenon to elucidate in *S. typhi murium* that the biochemical synthetic process of nutrition is governed by a series of many genes.

Further, Iseki and Sakai,³⁾ and Sakai and Iseki⁴⁾ clarified that in *Salmonella* group E *epsilon* phage participating in lysogenic conversion with regard to antigen 15 not only induced antigenic transformation but also transduced genetic characters of the strain.

Also in other bacteria than *Salmonella*, similar phenomena were observed: The induction of motility in *Bacillus anthracis* by phage lysate reported by Brown, Cherry, Moody, and Gordon,⁵⁾ and the exclusive transduction of the galactose-fermenting ability (gal⁺) by temperate phage *lambda* in the sensitive strain of *Escherichia coli* K-12, in which recombination takes place, reported by Morse, Lederberg, and Lederberg.⁶⁾⁷⁾ In this latter case, they reported, different from that of *Salmonella*, the strain in which transduction was performed was heterozygous with respect to the marker of gal⁺, which was dominant, and gal⁻ appeared only in 1 out of 10³ bacterial divisions.

Previously Kauffmann⁸⁾ carried out experiments on the transduction of flagellar antigen by adding phage of *S. typhi murium* to *S. paratyphi* A var. *durazzo*, and he observed that together with flagellar antigen i somatic antigen 1 was also transduced, conversing the antigenic structure from 2,12:a:- to 1,2,12:i:-. And we found out that *iota* phage which was the transforming agent of somatic antigen 1 in *Salmonella* also transduced the flagellar antigen and other genetic characters of the donor strain.

Materials and methods. 1) Phage used in transduction experi-

ments. The *iota* phages obtained from *S. paratyphi* A 1015 (S-1), *S. paratyphi* B 6617 (S-4), *S. paratyphi* B var. *odense* 9864 (S-8), *S. typhi* *murium* 4066 (S-10), *S. typhi* *murium* var. *copenhagen* 3146 (S-12), and *S. typhi* *murium* *masaki* (Masaki), all of which contain somatic antigen 1, were made to infect a streptomycin resistant strain (antigens 4,5,12:b:1,2, prototroph, SM^r) of *S. paratyphi* *kanbe* (Kanbe) and a streptomycin resistant strain (antigens 4,12:i:1,2, prototroph, SM^r) of *S. typhi* *murium* var. *copenhagen* 659 (S-11) to induce bacteriolysis. And the phage liquids which produced lytic plaques attaining the titer of 10⁹/cc were used for transduction. These phage liquids were respectively denoted by abbreviations i1(K), i4(K), i8(K), i10(K), i12(K), iM(K), i1(11), i4(11), i8(11), i10(11), i12(11), iM(11). Phage i1(K), for example, indicates that *iota* phage from S-1 is enhanced by infecting Kanbe SM^r strain. The degree of streptomycin resistance of the host strain was such that it could grow in a medium containing streptomycin at the rate of 100 mg/cc.

2) Strains used in experiments on the transduction of streptomycin resistance. Group A: *S. paratyphi* A 1015 (S-1), *S. paratyphi* A var. *durazzo* (S-2). Group B: *S. paratyphi* B 3006 (S-3), *S. paratyphi* B 6617 (S-4), *S. paratyphi* B var. *java* (S-5), *S. paratyphi* B *kanbe* (Kanbe), *S. paratyphi* B var. *odense* 9864 (S-8), *S. typhi* *murium* 1406 (S-9), *S. typhi* *murium* 4066 (S-10), *S. typhi* *murium* var. *copenhagen* 659 (S-11), *S. typhi* *murium* var. *copenhagen* 3146 (S-12), *S. typhi* *murium* (O-form) 3173 (S-13), *S. typhi* *murium* *masaki* (Masaki), *S. stanley* (S-15), *S. abortus equi* (S-26). Group D: *S. typhi* H 901 W (S-57), *S. enteritidis* (S-64), *S. sendai* (S-71), *S. dar-es-salaam* (S-72).

Each 0.5 cc of a 24-hour culture liquid of these strains and each 0.5 cc of the above-mentioned phage liquids were added to 1 cc of broth, and cultured at 37°C for 24 hours. Then, after washing with physiological saline solution, cells were inoculated into SM agar medium containing streptomycin at the rate of 5 mg/cc, and after 48 hours, grown bacteria were examined for the number of colonies, the presence or the absence of somatic antigen 1 and lysogenicity.

3) Experiments on the transduction of nutritional factor and streptomycin resistance. To S-3A₁ (cystineless (C⁻), streptomycin sensitive (SM^s)) and S-3A₂ (arginineless (A⁻), streptomycin sensitive (SM^s)), which were obtained by treating *S. paratyphi* B 3006 (S-3) with nitrogen mustard, was added phage liquid i12(K), and by the above described method, experiments on the transduction of nutritional factor and streptomycin resistance were carried out. In this case, as the selective medium, were employed minimum medium, streptomycin-added agar medium, and streptomycin-added minimum medium. The composition of the minimum medium was as follows: NH₄Cl, 5 g; NH₄NO₃,

1 g; Na_2SO_4 , 2 g; K_2HPO_4 , 3 g; KH_2PO_4 , 1 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g; glucose, 5 g; distilled water, 1000 cc; purified agar, 15 g.

4) Experiments on the transduction of flagellar antigen and streptomycin resistance. Phage liquid i12(K) was added to *S. typhi* H 901 W (S-57) (antigens 9,12:d:—, SM⁺) and cultured. As the selective medium the following two kinds were used: A 0.2% semisolid agar medium containing 1/100 its volume of anti-d serum which was prepared by absorbing anti-S-57 rabbit serum with heat-killed cells of S-57 or S-64 (1,9,12:g,m:—); the other was prepared by adding streptomycin to the above at the rate of 5 mg/cc. Each of these media was put into the test tube, and in the center of the media was set up a glass tube in which the above-mentioned strain was inoculated. After being cultured in the incubator for 10 days, bacteria which had grown to the surface of the medium outside the glass tube were transplanted to the agar plate, and then examined for the antigenic structure and streptomycin resistance.

5) Anti-phage serum. The rabbit was immunized with phage liquid i4(K), and the serum obtained was absorbed with *S. paratyphi* B *kanbe*.

Results. 1. Transduction of streptomycin resistance. As shown in the table, the results varied according to the combination of the strain and the phage.

When a strain without somatic antigen 1, for example, S-3, which is easily lysed and lysogenized with donor phage liquid i1(K), was used as a recipient, the transduction frequency of streptomycin resistance was 10^{-7} , and when the transduction occurred the strain was always turned lysogenic acquiring somatic antigen 1. A similar fact is generally observed in transduction experiments with *iota* phage and the strain which is easily lysogenizable with this phage. In such cases the transduction frequency is very high, and the strain after the transduction always comes to possess somatic antigen 1.

When the strains hardly lysogenizable with donor phage liquid i1(K) such strains as S-5 and S-13, were used as recipients for examination for the transduction of streptomycin resistance, the transduction was effected in some strains such as S-5, but it was difficult in others such as S-13. But the frequency was 10^{-8} , rather lower even in S-5 than when easily lysogenizable strains were used, and many in which the transduction was effected remained sensitive strains without being lysogenized and without having somatic antigen 1. In some cases, however, streptomycin resistance was transduced into S-5 and S-13 at the frequency of 10^{-7} by a phage liquid which hardly lysed or lysogenized these strains, for example, i1(11).

In this way, donor phage liquids i1(K) and i1(11) transduced

Transduction of streptomycin resistance by *iota* phage

Recipient strain			Iota phage liquid											
Group	Strain	Antigen 1	i1(K)	i4(K)	i8(K)	i10(K)	i12(K)	iM(K)	i1(11)	i4(11)	i8(11)	i10(11)	i12(11)	iM(11)
A	S-2	-	-	-	-	-	-	-	-	-	-	-	-	-
	S-3 Kanbe	-	++100	++100	++100	80	++100	++100	+	100	+	100	++100	++100
B	S-5	-	++100	++100	++100	80	++100	++100	++	100	++	100	++100	++100
	S-9	-	+	0	0	30	+	20	++	0	++	0	++	25
	S-11	-	-	±	60	-	++100	-	++	100	+	100	++100	++100
	S-13	-	+	100	-	-	++	100	++	++100	++100	++100	++100	++100
	S-15	-	-	-	-	-	+	100	++	0	++	0	++100	++50
	S-26	-	-	-	-	±	0	++100	++100	0	+	0	++100	++80
		-	-	-	-	+	100	±	+	0	+	0	++100	++80
		±	100	±	100	±	100	±	0	+	+	+	+	+
D	S-57	-	±	100	±	100	++80	++	++	60	++	40	++	++
	S-64	-	-	-	-	-	-	-	-	-	±	0	-	-
B	S-1	+	-	-	-	±	-	-	±	±	±	-	-	-
	S-4	+	-	-	-	-	±	-	-	-	-	-	-	-
	S-8	+	-	±	±	±	-	-	-	-	-	±	-	-
	S-10	+	-	-	-	-	-	-	-	-	-	-	-	-
	S-12	+	-	-	-	-	-	-	-	-	-	-	-	-
	Masaki	+	-	-	-	-	±	-	-	-	-	±	±	-
D	S-71	+	-	-	-	-	-	-	±	-	±	-	-	-
	S-72	+	-	-	-	-	-	-	-	-	-	-	-	-

Remarks: Frequency of transduction: ++, +, 10⁻⁶; ++, 10⁻⁷; ±, 10⁻⁸; ±, 10⁻⁹, and lower. Figures denote frequencies of antigenic transformation (%) concerning somatic antigen 1, occurring simultaneously with the transduction of streptomycin resistance.

streptomycin resistance into recipient strains at different frequencies, assumedly because these phages, though obtained from the same strain S-1, were multiplied in different host cells.

Also there were strains, such as S-2 and S-64, into which streptomycin resistance was not transduced by any *iota* phage.

Further, there were cases which can be considered intermediate between the above-mentioned transductions — into strains easily lysogenized by the phage, and into strains hardly lysogenized.

For example, when to recipient strain S-5 was added donor phage liquid i10(K) which partly lysogenized this strain, the frequency of the transduction of streptomycin resistance was 10^{-8} , and in 30% of the transduced cells lysogenization and lysogenic conversion with regard to somatic antigen 1 occurred. Further, when phage liquid i1(K) was used as the donor and S-8, which has somatic antigen 1, as the recipient, the transduction of streptomycin resistance was effected, but only at the frequency of 10^{-9} , lower than in the case of recipients which lack antigen 1.

That the above-mentioned transduction of streptomycin resistance was effected by *iota* phage was evident, since it was not observed when this phage was neutralized with anti-*iota* phage serum.

2. Transduction of nutritional factor and streptomycin resistance. From cystineless S-3 A₁ and arginineless S-3 A₂ which were treated with i12(K), prototrophs and streptomycin resistant strains were produced. And in the minimum medium and the streptomycin-added agar medium in which each of the above was cultured, bacterial colonies were formed at the frequency of about 10^{-7} , but in the streptomycin-added minimum medium no bacterial growth was observed. Moreover, the strain which grew in the streptomycin-added agar medium did not grow in the minimum medium, while that which grew in the latter did not in the former. This indicates that the phage generally transduces only one genetic character into one bacterial cell. However, in the case of the above-mentioned mutants of S-3, any cells in which either nutritional factor or streptomycin resistance was transduced had been lysogenized by *iota* phage, at the same time producing somatic antigen 1.

3. Transduction of flagellar antigen. Phage liquid i12(K) was used as the donor, and S-57 as the recipient strain. When the phage-treated S-57 was inoculated in the glass tube containing anti-d serum-added semisolid agar, the diffusion of bacteria was observed, but when it was inoculated into the glass tube containing semisolid agar to which were added streptomycin and anti-d serum, the diffusion was not visible. The investigation of the diffused bacteria revealed that the change of flagellar antigen from d to b had occurred in them, but that they were all streptomycin sensitive. Further, bacteria which

were transduced flagellar antigen had produced somatic antigen 1, so that the antigenic structure in them turned from 9,12:d:— into 1,9,12:b:—. The case in which concurrently with the transduction of flagellar antigen the transformation concerning somatic antigen 1 was performed by phage was also observed by Kauffmann.⁸⁾ This is explained as follows: Lysogenic conversion with regard to somatic antigen 1 is induced by *iota* phage, and concurrently the transduction of flagellar antigen is performed.

Summary. *Iota* phage, which induces lysogenic conversion with regard to somatic antigen 1 of *Salmonella* groups A, B, and D, can also transduce, like *epsilon* phage^{3,4)} in *Salmonella* group E, such genetic characters as streptomycin resistance, nutritional factor, and flagellar antigen separately into the recipient strain. The transduction of characters can occur regardless of whether the recipient strain is easily lysogenizable or hardly lysogenizable. In the former case, the transduced bacteria turn lysogenic, acquiring somatic antigen 1, while in the latter, even after the transduction of characters, they generally remained sensitive without somatic antigen 1. The transduction of characters is induced by *iota* phage sometimes also into recipient strains which possess somatic antigen 1, but at extremely lower frequencies.

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