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Different frequencies of cotransduction of *motC* and *H1* in *Salmonella*

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1. INTRODUCTION

In *Salmonella*, mutation in the *fla* (flagellation) genes, (*A*, *B*, *C*, *D*, *E*, *F*, *J*, and *K*), or *mot* (motility) genes, (*A*, *B*, and *C*), causes loss of bacterial movement; a *fla*$^-$ mutation results in absence of flagella and a *mot*$^-$ mutation causes flagellar paralysis. Some *fla*$^-$ and *mot*$^-$ mutants, when treated with phage P22 grown on motile strains having *H1* (the phase-1 flagellar antigen gene: Lederberg & Edwards, 1953) different from the recipients, produce motile transductants with the phase-1 flagellar antigen of the donor. This means that these *fla*$^-$ or *mot*$^-$ mutational sites are closely linked to *H1*, so that the chromosomal fragment transduced by a single phage P22 particle can carry the *fla*$^+$ or *mot*$^+$ allele of the donor together with its *H1* allele. By the frequencies of cotransduction with *H1*, many *fla*$^-$ and *mot*$^-$ sites have been mapped around *H1* (Joys & Stocker, 1963; Iino & Enomoto, 1966; Enomoto, 1966*a*). However, the sites mapped in each experiment cannot be compared with one another, because the donor differed from one experiment to the next and might have produced different frequencies of cotransduction. The *flaK* and *motC* genes used in this work are also cotransducible with *H1* and are arranged in the order, *motC-H1-flaK* (Enomoto, 1967). By transduction from various Salmonella serotypes to *flaK*$^-$ and *motC*$^-$ mutants of *S. typhimurium*, motile serotypic recombinants selected for the donor's *H1* allele as well as for *flaK*$^+$ or *motC*$^+$ can be isolated. In the present work, the frequency of cotransduction of *motC* with *H1* has been extensively studied, using several serotypic recombinants and various serotypes as donors and a *motC*$^-$ mutant as a recipient. The frequency of cotransduction varied with the donors, although they were serotypic recombinants with indistinguishable phase-1 flagellar antigens. This variability is thought to have two causes: (1) a difference in genetic homology of the chromosomes (at the molecular level) between donor and recipient; and (2) differences in genetic composition of the transducing fragments concerned. The first factor was previously described as the cause of the low frequency of integration of a given marker in the transduction tests between *S. typhimurium* and *S. typhimurium–Escherichia coli* hybrids or *S. typhimurium–S. montevideo* hybrids (Demerec & Ohta, 1964; Dmerec & New, 1965; Eisenstark, 1965; Glatzer, Labrie & Armstrong, 1966). The second has been described by Pearce & Stocker (1965), Roth & Hartman (1965), and Enomoto (1967).

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2. MATERIALS AND METHODS

(i) *Bacterial and phage strains*

The bacterial strains are described in Table 1. They all, except SJ11 belonging to somatic group D1, belong to group B of the Kauffmann–White table (Kauffmann, 1964). Strain SJ916, an *H1* mutant with an altered form of flagellar antigen *i*, was obtained by picking a swarm of *S. typhimurium* wild-type strain, TM2, expressing wild-type phase-1 antigen *i*, inoculated on NGA medium containing sufficient anti-*i* and anti-*1.2* sera to inhibit spread of wild-type cells. Cells expressing the altered phase-1 antigen were indistinguishable from wild-type cells in their mode of locomotion under the microscope and speed of spreading on NGA

Table 1. *Bacterial strains*

Strain (mutant no.)	Flagellar antigen		Descriptions
	Phase-1	Phase-2	
SJ11	*gp*	—	*Salmonella dublin*, wild type
SJ241	*a*	(*enx*)	*S. abortus-equi*, phase-1 monophasic strain
SW803	*b*	*enx*	*S. abony*, wild type
SW1391	*b*	*enx*	*S. abony*, Hfr of SW803, requiring methionine and aromatic amino acid
TM2	*i*	*1.2*	*S. typhimurium*, wild type
SJ916	*i'*	*1.2*	Serological mutant of TM2
SJ448 (*motC244*)	*i*	*1.2*	Paralysed mutant of TM2
SJ799 (*flaK48*)	(*i*)	(*1.2*)	Non-flagellated mutant of TM2
SJ730 (*motC244 flaK48*)	(*i*)	(*1.2*)	Paralysed and non-flagellated mutant of TM2
SJ646 (*hisE11*)	*i*	*1.2*	*S. typhimurium, requiring histidine*

Flagellar antigen in parenthesis is not expressed.

medium. They were agglutinated to a titre of 1300 by anti-*i* (wild type) serum prepared against SJ847 (*S. abortus-equi* SJ241 with *H1i* from *S. typhimurium* TM2) which agglutinated TM2 to a titre of 20000. This serum, when fully absorbed with SJ916, still agglutinated TM2 to a titre of 10000. The mutants *motC244* (non-motile though flagellate) and *flaK48* (non-flagellate) were used as recipients in transduction tests with donors of various serotypes to obtain serotypic recombinants. *motC244* was also used for studying the frequency of cotransduction. *flaK48* was obtained by transduction of the mutant gene *flaK48* of a double mutant, *motC244 flaK48*, to TM2. The serotypic recombinants, obtained by transduction or by conjugation using Hfr strain SW1391 and later used as donors for studying the frequency of cotransduction of *H1* with *motC*, were described in the latter section. *hisE11* was obtained from Dr P. E. Hartman, Johns Hopkins University, Maryland, U.S.A.

Phage P22 (Zinder & Lederberg, 1952) was propagated on donor strains by a modified agar-layer method (Adams, 1959). Lysates were centrifuged at 3000 *g* for 15 min, and the supernatants sterilized by chloroform and used for transduction.

(ii) *Media*

Nutrient broth, nutrient agar (NA), semi-solid nutrient gelatin agar (NGA) and semi-solid minimal gelatin agar (MGA) have been described previously (Stocker, Zinder & Lederberg, 1953; Enomoto, 1966*a*, *b*). Anti-flagellar sera with titres of about 20000 were prepared in this laboratory and added to NGA medium at a final concentration of about 0·1 % (v/v), which completely inhibited the production of swarms by strains with the corresponding flagellar antigens.

(iii) *Transduction and scoring of cotransductants*

The mutant *motC244* was used as recipient throughout the experiments on the frequency of cotransduction. A broth culture (5×10^8 to 1×10^9/ml.) of the recipient was grown from a single colony expressing phase-1 flagellar antigen *i* and mixed with an equal volume of phage suspension at an input ratio about 5. The mixture was spread on NGA plates after appropriate dilution and incubated for 8 h at 37 °C. Motile transductants appearing as swarms were isolated on NA, and their flagellar antigens typed by slide agglutination tests with antisera for the phase-1 flagellar antigens of the donor and the recipient, respectively. In the transduction test with SJ916 which has a mutant *i* antigen, swarms were stabbed in NGA plates containing anti-*i* (wild type) and anti-*1.2* sera and those clones spreading as swarms were regarded as transductants having the donor's flagellar antigen. The percentage of motile transductants showing the phase-1 flagellar antigen of the donor was taken as the frequency of cotransduction of *motC* and *H1*.

3. RESULTS AND DISCUSSION

(i) *Frequency of cotransduction of* motC *and* H1 *using various serotypes as donors*

Transductions were carried out from the motile wild-type strains, *S. abortus-equi* SJ241, *S. abony* SW803, and *S. dublin* SJ11, to a paralysed mutant of *S. typhimurium motC244*. SJ916, an *H1* mutant of *S. typhimurium* TM2, was used as control for the donors. As shown in Table 2, the frequency of cotransduction of *motC* with *H1* varied from 7 % to 52 %. This large variation should be interpreted with two possible factors in mind: one is the difference in homology (at the level of the base pair sequence of deoxyribonucleic acid) between the *S. typhimurium* chromosome of the recipient and the fragment from the donor; and the other is the variation in genetic composition of transducing fragments carrying *motC*, which will be found among P22 lysates of various Salmonella species; for instance, the variation in the ratio of the fragments carrying both *motC*$^+$ and *H1* to those carrying only *motC*$^+$. Transduction between strains derived from TM2 shows that 93 % of transducing fragments carrying *motC*$^+$ also carry *H1* and end between *H1* and *flaK* (Enomoto, 1967). In transduction with SJ916, the donor and recipient are identical except for the mutations in *motC* and *H1*, and no difference in homology exists. Consequently, the recombination frequency within a

given chromosomal region is in proportion to the size of the region. The observed frequency of 52% therefore suggests that the distance between *motC* and *H1* (region II in Fig. 1) is roughly equal to that between *H1* and the end of the fragment concerned (region III). Supposing the chromosomal fragments carried by P22 particles which are propagated on various salmonella species to have the same genetic composition as that of *S. typhimurium*, the lower frequencies obtained from the interspecific transductions suggest that the homology between the donated fragment and the recipient chromosome of *S. typhimurium* is less in region III than

Table 2. *Frequency of cotransduction of* motC *with* H1 *in experiments using several serotypes as donors*

(Methods of transduction and scoring of *H1*-cotransductants are described in the Materials and Methods.)

Donor	*H1*	No. of transductants tested	No. of donor type	*H1*-cotransduction %	*H1*-cotransduction 95% confid. (± %)	Frequency of transduction per 10^5 p.f.u.*
SJ916	*i'*	684	358	52·3	1·9	0·52
SJ241	*a*	293	21	7·2	3·0	0·0024
SW803	*b*	356	110	30·9	4·8	0·036
SJ11	*gp*	202	39	19·3	5·4	26·4

* The number of plaque-forming units of each donor was counted on *S. typhimurium* TM2.

Table 3. *Frequency of cotransduction using motile recombinants as donors*

(Donors were obtained by mating *S. abony* Hfr SW1391 with *S. typhimurium motC244 flaK48*. Selection was made for *motC*+, *H1b*, and *flaK*+ from the donor and *met*+ from the recipient. Details are given in Enomoto (1966*b*). Motile recombinants spreading as swarms on MGA medium were picked and used as donors for transduction tests to *motC244*. Scoring of *H1*-cotransduction is described in the Materials and Methods.)

Donor	No. of transductants tested	No. of donor type	*H1*-cotransduction %	*H1*-cotransduction 95% confid. (± %)
SJ902	623	95	15·2	2·8
SJ904	589	72	12·2	2·6
SJ905	619	71	11·5	2·5
SJ908	504	73	14·5	3·1

in II; furthermore, the difference between region II and III is most marked in *S. abortus-equi*, since, if the homology was the same for II and III, the frequency should be around 52%. On the other hand, if the transducing fragments carrying *motC*+ in the lysate of each species differ in genetic composition from those of *S. typhimurium*, the lower frequencies could mean either that region III of the transduced fragment is shorter in these species than in SJ916, or that the fragments carrying only *motC*+ are abundant, due to an increased frequency of breaks between *motC* and *H1*.

Table 3 shows the frequency of cotransduction in experiments using as donors the motile recombinants obtained by mating *S. abony* Hfr SW1391 with the double mutant of *S. typhimurium*, *motC244 flaK48*. These recombinants were selected for *motC*$^+$, *flaK*$^+$ and *H1b* of the donor, and consequently received from the donor at least the chromosomal region lying between *motC* and *flaK*. The frequencies of cotransduction were from 12 % to 15 %, very different from the frequency of 52 % obtained with SJ916. This suggests that the difference in homology between the fragment of *S. abony* and the *S. typhimurium* chromosome is more marked in region III than II, because most of the chromosome of these recombinants orginates from *S. typhimurium* (the region carrying the *motC*, *flaK*, and *H1b* is *S. abony* in origin), so that the transducing particles prepared from them are presumed to have the same genetic composition as those of *S. typhimurium* SJ916. These frequencies also differ from the value of 31 % obtained with SW803, a parental strain of SW1391. The difference between the two is thought to arise from the difference in genetic composition of the transducing fragment concerned, e.g. region III from SW803 is longer than that from the recombinants or from SJ916, because regions II and III from SW1391 should have the same genetic homology as those of SW803 from which it was derived.

(ii) *Frequency of cotransduction using serotypic recombinants as donors*

In order to diminish differences in genetic composition of the transducing fragments derived from different species, the chromosomal region carrying the *H1*

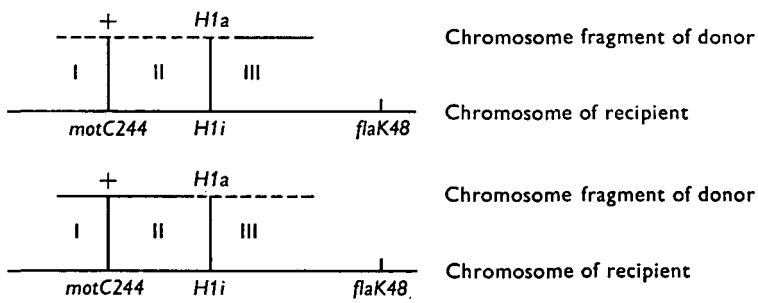

Fig. 1. Transduction from serotypic recombinants to *S. typhimurium motC244*. The donor in the top cross was obtained by selecting the *motC*$^+$ and *H1* genes of the donor in the transduction from *Salmonella* sero-types to *motC244*. The donor in the bottom cross was selected for *flaK*$^+$ and *H1* of the donor in the transduction from Salmonella serotypes to *flaK48*. Dotted lines show the non-homologous region transduced from the donor. Most transducing fragments carrying *motC*$^+$ have an end between *H1* and *flaK*.

gene was transduced from the three donors, SJ241, SW803 and SJ11, to *S. typhimurium*, using phage P22H4, the v1 mutant of Zinder (1958). Transductions were carried out from these donors to *motC244* expressing the phase-1 flagellar antigen and to *flaK48* with latent phase-1, using NGA medium containing anti-*i* and anti-*1.2* sera. Spreading swarms—that is, cotransductants of *H1* with *motC*$^+$ or *flaK*$^+$ —were picked and clones sensitive to P22 isolated. The serotypic recombinants

Table 4. *Frequency of* H1*-cotransduction and transduction using serotypic recombinants as donors*

Donor	Selected gene	No. of transductants tested	Donor type	Frequency of *H1*-cotransduction		Frequency of transduction*			
						motC244 as recipient		*hisE11* as recipient	
				%	95 % confid. (± %)	No.	%	No.	%
SJ844	*H1 motC* of SJ11	236	75	31·8	5·9	2·13	41·3	6·04	145·0
SJ832	*H1 motC* of SJ11	173	76	43·9	7·4	1·44	27·9	3·58	86·0
SJ840	*H1 flaK* of SJ11	199	9	4·5	2·9	4·66	90·2	4·44	106·5
SJ841	*H1 flaK* of SJ11	194	41	21·1	5·7	0·975	18·9	4·67	112·2
SJ842	*H1 flaK* of SJ11	290	46	15·9	4·2	2·02	39·1	4·49	108·0
SJ843	*H1 flaK* of SJ11	200	20	10·0	4·2	1·64	31·8	5·13	123·4
SJ865	*H1 motC* of SJ241	320	55	17·2	4·2	1·88	36·4	4·60	110·5
SJ866	*H1 motC* of SJ241	491	36	7·3	2·3	1·45	28·1	2·68	64·5
SJ878	*H1 flaK* of SJ241	397	11	2·8	1·6	4·65	90·0	3·86	92·8
SJ879	*H1 flaK* of SJ241	397	32	8·1	2·7	4·77	92·3	5·19	129·5
SJ867	*H1 motC* of SW803	400	64	16·0	3·6	1·62	31·4	5·13	123·4
SJ868	*H1 motC* of SW803	343	79	23·0	4·4	0·960	18·6	3·28	78·9
SJ875	*H1 flaK* of SW803	364	13	3·6	1·9	3·08	59·6	4·88	117·2
SJ877	*H1 flaK* of SW803	254	30	11·8	4·0	0·743	14·4	2·12	51·0
SJ916	—	684	358	52·3	1·9	5·17	100·0	4·16	100·0

* Expressed as the number of transductants produced by the lysate containing 10^6 plaque-forming units.

obtained from *motC244* presumably received at least the chromosomal region lying between *H1* and *motC* from the donor (termed '*motC*-transductants'), while those from *flaK48* received the region between *H1* and *flaK* ('*flaK*-transductants'). Both types of transductant were used as donors for tests with *motC244* (Fig. 1), and the frequency of cotransduction of *motC* with *H1* examined. The results are shown in Table 4, where the transduction frequencies obtained on crossing each donor to *motC244* and to *hisE11* are also given. The frequency of cotransduction varied considerably with donors although they were phenotypically indistinguishable: from 4·5% to 43·9% with the donors obtained from SJ11, from 2·8% to 17·2% with SJ241, and from 3·6% to 23·0% with SW803. The frequency of cotransduction was generally higher in experiments using *motC*-transductants than *flaK*-transductants. Thus, in the crosses with *motC*-transductants, the reduced homology in region II presumably cause a decreased rate of recombination, resulting in a relative increase in cotransduction frequency with *H1* (the top cross in Fig. 1). With *flaK*-transductants, region III showed the reduced homology, resulting in a relative decrease in cotransduction frequency (the bottom cross in Fig. 1). With the *motC*-transductants, the frequency of cotransduction is expected to be more than 50%, which was obtained from the transduction with SJ916, assuming that the chromosomal region showing the reduced homology is restricted to region II. However, all the frequencies obtained from the *motC*-transductants were less than 50%. This suggests that the chromosomal portion originating from the donor is not restricted to region II but extends also to region III, in which the difference in homology between the donor and the recipient is more marked than in II, so that recombination resulting in cotransduction with *H1* is much reduced.

The frequencies in transduction tests from each donor to *hisE11*, used as a control for the recipient, were from 50% to 150% (taking the frequency with SJ916 as 100%) and the difference between them is negligible; while in transduction to *motC244* many were less than 50%. It is of interest that donors giving frequencies of more than 50%, such as SJ840, SJ878 and SJ875, are *flaK*-transductants and show a lower frequency of cotransduction with *H1*. In these strains, it is supposed that the heterogeneous chromosomal region derived from other species is restricted to the narrow region containing *flaK*$^+$ and *H1* and that most of region II is from *S. typhimurium*. Therefore, recombination occurs with normal frequency in region II, resulting in a relative decrease in the frequency of cotransduction with *H1* and in transduction frequencies not differing significantly from the control.

SUMMARY

The frequency of cotransduction of *motC* and *H1* in Salmonella has been investigated, using four Salmonella serotypes and many serotypic recombinants as donors and *S. typhimurium motC* mutant as recipient. The frequency varied with the four serotypes from 7% to 52%. It is suggested that the difference in frequency arises from not only differences in genetic homology between the chromosome of the recipient and the fragment from the donor, but also from differences in genetic composition of the chromosome fragments carried by the phage. The

frequency of serotypic recombinants selected for *motC*$^+$ and *H1* gene of the donor is generally higher than with recombinants selected for *flaK*$^+$ and *H1*. The difference in genetic homology between *S. typhimurium* and other species is more marked in the region between *H1* and *flaK* than between *motC* and *H1*.

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