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Genetic Basis of Vi Antigen Expression in *Salmonella paratyphi* C

NORMA J. SNELLINGS,* E. M. JOHNSON, AND L. S. BARON

Department of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20012

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Analysis of hybrids formed in a cross between a *Salmonella paratyphi* C Hfr and an *S. typhimurium* recipient indicated that the structural genetic determinants of the *S. paratyphi* C Vi antigen are located closely adjacent to the *mel* determinant, between this marker and *purA*. A similar location was indicated for the structural genetic determinants of the *S. typhi* Vi antigen (the *viaB* locus) by the results of a mating in which a hybrid *S. typhimurium* Hfr bearing the *S. typhi viaB* determinants was used to transfer these genes to an *S. typhimurium* recipient. Mating experiments with a Vi-antigen-expressing *S. typhi* Hfr and an *S. typhimurium* hybrid recipient expressing the Vi antigen of *S. paratyphi* C yielded no recombinants in which loss of Vi antigen expression occurred, indicating that the chromosomal locus occupied by the genetic determinants of the *S. paratyphi* C Vi antigen is the same one at which, in *S. typhi*, the *viaB* genes reside. Introduction of a mutant *S. typhi viaA* gene into an *S. typhimurium* hybrid expressing the Vi antigen, as the consequence of prior receipt of the *S. paratyphi* C *viaB* determinants, resulted in that hybrid's loss of Vi antigen expression, demonstrating that the *viaA* determinant plays a role in Vi antigen expression in *S. paratyphi* C, as well as in *S. typhi*. Although the percentages of coinheritance of the *viaB* and *mel* determinants in the mating experiments suggested that their linkage is sufficiently close to allow cotransduction by P22, attempts to accomplish this with lysates prepared on *S. typhimurium* hybrids expressing either *S. typhi* or *S. paratyphi* C *viaB* determinants were not successful.

The antigen termed Vi is expressed by several organisms of the family *Enterobacteriaceae*, but only in *Salmonella typhi* has the genetic basis of this expression been examined (3, 5, 6). There, determinants of the antigen occupy two widely separated chromosomal gene loci, designated *viaA* and *viaB*. The *S. typhi viaB* locus, situated near the determinant of adenine biosynthesis, *purA*, is occupied by genes which, when introduced into *S. typhimurium* or *Escherichia coli*, result in the expression of the Vi antigen in those organisms. It therefore appears to be the site at which the primary structural determinants of the antigen are located. The *viaA* locus is situated near the gene determining histidine biosynthesis, *his*. Mutation of a gene at the *viaA* locus of *S. typhi* results in loss of Vi antigen expression even though the genes at the *viaB* locus remain functional. The specific function of the *viaA* gene is not known, but this determinant is also present in other enteric bacteria, such as *S. typhimurium* (5) and *E. coli* (3), which do not normally express the Vi antigen. Thus, introduction of the native, functional *viaA* locus of *S. typhimurium* or *E. coli* into a *viaA* mutant of *S. typhi* restores that organism's ability to express the antigen.

In addition to its occurrence in *S. typhi*, the Vi antigen is found also in strains of *S. dublin*, in *S. hershfeldii* (*S. paratyphi* C), and in certain serotypes of *Citrobacter*. Although serologically identical, the antigens expressed by these organisms appear to differ among each other in varying degrees, both chemically and immunologically (2). Nevertheless, it seemed likely to us that their genetic determinants should be related to each other in the same way as the genetic determinants of the diverse somatic (8) and flagellar (7) antigens expressed among the *Enterobacteriaceae*, i.e., as members of a series of allelic genes occupying the same chromosomal locus in each organism. Having previously adapted a strain of *S. paratyphi* C to serve as an Hfr donor (E. M. Johnson, unpublished data), we were afforded the opportunity of examining this proposition, at least with respect to the Vi antigen determinants of *S. para-*

typhi C and *S. typhi*. In the present paper we report the results of our investigation of the genetic basis of Vi antigen expression in *S. paratyphi* C.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains employed in this study are described in Table 1. The *S. paratyphi* C Hfr and the two *S. typhi* Hfr strains were constructed by transfer of the F-linked terminal marker, Lac^+ (of *E. coli* origin) of SC-19 (4), and have, therefore, the same chromosome transfer orientation as that Hfr; i.e., *proA* is the lead marker and *lac* is the last marker transferred ahead of F.

Media. Minimal medium consisted of 320 ml of distilled water to which 80 ml of minimal salts solution containing K_2HPO_4 (70 g/liter), KH_2PO_4 (20 g/liter), NH_2SO_4 (10 g/liter), and $MgSO_4$ (0.5 g/liter) was added. The medium was solidified by the addition of a solution of separately autoclaved Noble agar (14 g in 400 ml of distilled water). Essential amino acids were added at a final concentration of 25 μg/ml each. In crosses in which the ability to utilize melibiose or lactose was the selected genetic trait, 0.4% of the carbohydrate was added as the sole carbon source. When the selected trait was an amino acid or a purine, glucose (0.4%) served as the sole carbon source, and the required growth factor was omitted from the medium. Streptomycin sulfate at a concentration of 625 μg/ml was used as a counterselective agent in crosses involving strains WR4060 and WR4000.

Technique of genetic crosses. Donor and recipient strains grown overnight in Penassay broth (Difco) at 37°C were centrifuged and resuspended in fresh Penassay broth so that a recipient concentration of approximately 10^9 cells per ml and a donor concentration of approximately 10^8 cells per ml were obtained. Adjusted donor and recipient suspensions (2.0 ml each) were mixed in a 50-ml Erlenmeyer flask and incubated at 37°C for 2 h. The mating suspension was then plated on minimal selective medium (0.1 ml/plate) either undiluted or in 10-fold dilutions depending on the expected frequency of recombination.

Biochemical and serological analysis of recombinants. Recombinant clones were purified by streaking on minimal medium of the same composition as that used in their selection. Purified clones were tested for melibiose utilization on MacConkey agar indicator medium containing 1% melibiose. Unselected inheritance of the Met^+ trait was determined by the ability of the hybrid to grow on minimal medium lacking methionine. The presence of Vi was determined by slide agglutination in antiserum prepared against *Citrobacter ballerup*.

Transduction. Phage P22 was grown by means of confluent lysis on the bacterial host strain to a titer of 10^{11} plaque-forming units (PFU) per ml. To 1 ml of the sterile phage lysate (diluted 1:10) was added 9 ml of an overnight Penassay broth culture of the recipient organism, making the final phage concentration 10^9 PFU/ml. After a 20-min incubation at 37°C, the cells were centrifuged, resuspended in 1 ml of Penassay broth, and plated on the appropriate selective medium (0.1 ml/plate). When melibiose utilization was the selected character, recipient concentrations of up to 5×10^{10} cells per ml and phage concentrations of up to 10^{11} PFU/ml were used.

RESULTS

Investigation of the *viaB* chromosomal region as a possible location of the structural determinants of the Vi antigen of *S. paratyphi* C. The *S. typhi viaB* locus is situated between the *metA* and *purA* loci, somewhat more closely linked to *purA* than to *metA* (5). Thus, it would be expected to occupy a position very close to the determinants of melibiose utilization (*mel*), genes not previously mapped in *Salmonella* but located on the *Escherichia coli* chromosome map (1) near min 92 (Fig. 1). Since correspond-

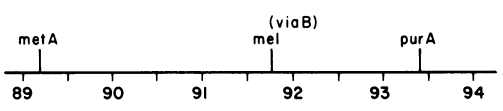

FIG. 1. *Segment of the bacterial chromosome map showing the approximate position of the S. typhi viaB locus relative to the locations of its metA, mel, and purA genes. The positions of the latter three determinants are depicted here in the time units derived from mapping studies with the corresponding markers of E. coli K-12 (1) but are considered reflective of the similar chromosomal locations of these genes in Salmonella.*

TABLE 1. *Bacterial strains*

Species	Strain	Sex	Relevant genetic characters	Source or derivation
S. typhimurium	SC-19	Hfr	*thr*	T. Miyake (9)
S. typhi	WR4000	Hfr	*cys trp*	From strain 643 by mating with SC-19 (4)
S. typhi	WR4013	Hfr	*cys trp viaA str*	From strain 643 WS^r by mating with SC-19
S. paratyphi C	WR4060	Hfr	*thy*	From strain ETS-32 of P. R. Edwards by mating with SC-19
S. typhimurium	WR5001	F^-	*his metA mel str*	From SU96 of K. E. Sanderson by mutation
S. typhimurium	WR5015	F^-	*purA str*	From PurA56 of K. E. Sanderson by mutation

ing genes almost invariably occupy similar locations in these related organisms, we think it safe to assume that the *E. coli* genetic segment depicted in Fig. 1 also reflects the locations of these genes on the *Salmonella* chromosome.

On the assumption that the structural genetic determinants of the *S. paratyphi* C Vi antigen would occupy the same chromosomal locus in that organism as in *S. typhi*, we mated the *S. paratyphi* C Hfr, WR4060, with the *S. typhimurium* recipient, WR5001, selecting for hybrids receiving the *mel* marker of the donor. Not surprisingly, Vi antigen expression occurred in 72 of 100 Mel$^+$ selected hybrids. The neighboring *metA* donor marker was inherited by only 18% of them. When the *metA* gene was used as the selected marker in this cross, 20% of the hybrids inherited the Vi antigen determinant and 27% inherited the donor *mel* marker. Of the 28 Met$^+$ selected hybrids that expressed either the *mel* or *via* determinants, 19 expressed both of those markers. These results, as well as the nature and number of the hybrid classes formed in these matings, are shown in Table 2 and are indicative of a very close linkage of the *mel* and *via* genes. The appearance of eight *metA mel* hybrids, as opposed to only one *metA via* hybrid among those selected for receipt of the donor *metA* gene, suggests that the gene order is *metA mel via*.

At this point, we had no doubt that the newly mapped *S. paratyphi* C *via* gene(s) and the previously mapped *S. typhi* determinants occupied a common locus, *viaB*, in each organism. We expected, therefore, that the *S. typhi* Hfr, WR4000, would behave as a *viaB* gene donor in much the same way as did the *S. paratyphi* C Hfr, WR4060. Surprisingly, however, such was not the case. Only 27 of 100 *S. typhimurium* WR5001 hybrids selected for receipt of the *S. typhi* WR4000 *mel* marker expressed the Vi antigen, a sharp contrast with the 72% inheritance of the *S. paratyphi* C *via* determinant with this marker. Furthermore, although 25 of 100 Met$^+$ selected hybrids from this cross inherited the donor *mel* marker, only 7 of these also expressed the Vi antigen. These results and the hybrid classes formed in these matings are shown in Table 3. Taken at face value, they do not contradict the gene order *metA mel viaB*, but they do appear to indicate that the linkage of the *mel* and *viaB* loci is much less close than is the case with the *mel* and *via* determinants of *S. paratyphi* C.

TABLE 2. *Unselected marker inheritance of hybrids formed in crosses between S. paratyphi C Hfr WR4060 and S. typhimurium recipient WR5001*[a]

Donor marker selected	Hybrid class	No.	Summation of unselected marker inheritance (%)
mel	*mel via*	56	*via*, 72
	mel	26	
	mel metA via	16	*metA*, 18
	mel metA	2	
metA	*metA*	72	*mel*, 27
	metA mel via	19	
	metA mel	8	*via*, 20
	metA via	1	

[a] Markers listed are those inherited from the Hfr strain.

Although acknowledging the possibility that the *S. paratyphi* C and *S. typhi* Vi antigen determinants might not be allelic genes, we considered it more likely that some aberrancy in the recombination of *S. typhi* and *S. typhimurium* chromosomes near the *viaB* locus was responsible for a lower frequency of *viaB* gene inheritance than would be expected from their assumed proximity to the *mel* locus. We therefore converted one of the *S. typhimurium* WR5001 hybrids expressing the *S. typhi metA*, *mel*, and *viaB* genes into an Hfr strain, introducing *lac* and F by remating with *S. typhi* WR4000. This hybrid *S. typhimurium* Hfr was then used to transfer its *S. typhi metA* and *mel* genes to an *S. typhimurium* WR5001 recipient into which a His$^+$ marker (from WR4000) had been previously introduced (to make possible the counterselection of the His$^-$ Hfr). Our rationale was that the homology, in this instance, between donor and recipient chromosomes in the region adjacent to the *metA mel viaB* segment might allow an inheritance pattern that would correctly reflect the locations of these genes.

The results of the matings between the *S. typhimurium* hybrid *viaB* donor and the His$^+$ WR5001 recipient are presented in Table 4. Whereas the unselected inheritance of the *S. typhi metA* and *mel* determinants in these crosses did not differ significantly from that previously observed with *S. typhi* WR4000 as the donor, a marked increase was seen in the unselected inheritance of *viaB*. Inheritance of *viaB* occurred in 87% of the Mel$^+$ selected hybrids and in 25% of the hybrids selected for receipt of the *metA* determinant. Furthermore, of the 30 Met$^+$ selected hybrids that also acquired either *mel* or *viaB*, 24 inherited both of those markers. Thus, these data indicate a linkage of the *S. typhi viaB* and *mel* determinants on the order of that observed with the *via* and *mel* genes of *S. paratyphi* C. Reflection of the *metA mel viaB* gene order in this cross, however, is not as strong as was the case in the

TABLE 3. *Unselected marker inheritance of hybrids formed in crosses between S. typhi Hfr WR4000 and S. typhimurium recipient WR5001*[a]

Donor marker selected	Hybrid class	No.	Summation of unselected marker inheritance (%)
mel	*mel*	67	
	mel viaB	27	*viaB*, 27
	mel metA	6	*metA*, 6
metA	*metA*	75	*mel*, 25
	metA mel	18	*viaB*, 7
	metA mel viaB	7	

[a] Markers listed are those inherited from the Hfr strain.

TABLE 4. *Unselected marker inheritance of hybrids formed in crosses between a hybrid S. typhimurium Hfr strain bearing the S. typhi viaB genes and a histidine-independent hybrid of S. typhimurium recipient WR5001*[a]

Donor marker selected	Hybrid class	No.	Summation of unselected marker inheritance (%)
mel	*mel viaB*	82	
	mel	11	*viaB*, 87
	mel metA viaB	5	*metA*, 7
	mel metA	2	
metA	*metA*	70	
	metA mel viaB	24	*mel*, 28
	metA mel	4	*viaB*, 26
	metA viaB	2	

[a] Markers listed are those inherited from the Hfr strain.

cross with the *S. paratyphi* C Hfr, since the presumed quadrupal crossover class (*metA viaB*) appears in two of only six Met^+ selected hybrids in which the *viaB* and *mel* genes were not inherited together.

We then employed the lone Met^+, Vi antigen-expressing, Mel^+ *S. typhimurium* WR5001 hybrid fathered by the *S. paratyphi* C Hfr WR4060 as the recipient in a mating with *S. typhi* Hfr WR4000. With each parent in this cross expressing a Vi antigen of different genetic origin, we reasoned that if their *via* determinants did not occupy a common locus, loss of Vi antigen expression should be observed occasionally among the Mel^+ selected hybrids. In other words, some hybrids would be expected to lose their *S. paratyphi* C *via* genes by recombination while also failing to inherit the *viaB* determinants of the *S. typhi* donor, particularly in view of the infrequency with which Hfr WR4000 appears able to donate those determinants. However, in examining over 500 Mel^+ selected hybrids from this cross, we did not observe any in which loss of Vi antigen expression occurred. The indication, therefore, is that the structural determinants of the Vi antigen of *S. paratyphi* C occupy the same chromosomal locus in that organism as the one at which, in *S. typhi*, the *viaB* genes reside.

Examination of the role of the *viaA* gene in Vi antigen expression by *S. paratyphi* C. A functional *viaA* determinant, situated near the *his* locus, has been demonstrated in both *S. typhimurium* and *E. coli*, which do not normally express the Vi antigen. Therefore, we would not think it remarkable that this determinant should be present and functional also in *S. paratyphi* C. However, we were not able to demonstrate this directly (by substitution of the mutant *viaA* gene of *S. typhi* with the functional *S. paratyphi* C allele), because the *S. paratyphi* C Hfr proved incapable of transferring its relatively distal *his* marker to *S. typhi* recipients. Surprisingly, transfer frequencies of *S. paratyphi* C Hfr WR4060 genes were considerably lower in matings with *S. typhi* recipients than in crosses with *S. typhimurium* WR5001. Thus, transfer of the WR4060 *metA* determinant to *S. typhimurium* WR5001 occurred at a frequency of 10^{-4} per donor cell, whereas its transfer to an *S. typhi* recipient occurred at only 5×10^{-7}.

We were able to demonstrate a role for the *viaA* determinant in expression of the *S. paratyphi* C Vi antigen by using a non-Vi-expressing *S. typhi* Hfr, WR4013, with functional *viaB* genes but with a mutant *viaA* determinant, to transfer *his* and the mutant *viaA* gene to an *S. typhimurium* WR5001 Mel^+ hybrid expressing the *S. paratyphi* C *viaB* genes. In this cross, 25% of the His^+ selected hybrids lost their ability to express the Vi antigen as the result of inheriting the mutant *viaA* gene of the *S. typhi* donor. A similar result (23% loss of Vi antigen expression) was observed when a Mel^+ *S. typhimurium* WR5001 hybrid expressing the *S. typhi viaB* genes served as the recipient with this Hfr.

Attempts to cotransduce the *viaB* determinants with *mel* and *purA*. We expected from its behavior in the conjugation experiments that the *viaB* locus would be situated close enough to *mel* to allow its cotransduction with that marker. We were hopeful, therefore, that the frequency of cotransduction with *mel*, and possibly with *purA*, would further define the location of *viaB* with respect to those markers. However, whereas the *purA* determinant was readily transduced to the *S. typhimurium* recipient strain, WR5015, with P22 lysates prepared from *S. typhimurium* WR5001 hybrids

expressing either the *S. typhi* or *S. paratyphi* C Vi antigen (10^{-7}/PFU), the *mel* determinant proved an especially difficult selected marker in transduction experiments. Working with lysates of up to 10^{11} PFU per ml and recipient (*S. typhimurium* WR5001) concentrations as high as possible within the limits of marker revertability, we obtained only one to five presumed Mel^+ transductants per 0.1 ml of the mixture plated. We examined for Vi antigen expression over 100 of these presumed transductants obtained with lysates prepared on WR5001 hybrids expressing the *S. paratyphi* C *viaB* determinants and a similar number of those obtained with lysates prepared on hybrids expressing the *S. typhi viaB* genes, but in no instance was Vi antigen expression observed. However, the very low frequency at which these presumed transductants were obtained leaves us in doubt as to whether any significance should be ascribed to this result. There was no apparent abnormality, of course, in the case of the *purA* selected transductants, but neither, in examining over 500 of them (300 by lysates prepared on *S. paratyphi* C-fathered, Vi-expressing hybrids; 200 by lysates prepared on *S. typhi*-fathered hybrids), did we observe any cotransduction of the *viaB* determinants.

DISCUSSION

If one takes the Vi antigen of *S. typhi* as the basis for comparison, the Vi antigen of *S. paratyphi* C would appear, from a chemical and immunological point of view, to be one of the least related among the antigens sharing that name. Edwards and Ewing (2) pointed out, from the results of immunoelectrophoretic analysis, that of the six identified, cathodically migrating fractions of the *S. typhi* Vi antigen, four are shared by the Vi antigen of the *Citrobacter* serotypes, whereas the *S. paratyphi* C Vi antigen consists of just two fractions that are related to two of those shared by the *S. typhi* and *Citrobacter* antigens. Nevertheless, from the genetic point of view, the findings of our present study indicate that the Vi antigen of *S. paratyphi* C is indeed a legitimate relative of the Vi antigen of *S. typhi*. Except for the unexpectedly low inheritance of the *viaB* genes when *S. typhi* served as the donor, our data point to an allelic relationship of the structural genetic determinants of the Vi antigens of *S. paratyphi* C and *S. typhi* at the chromosomal locus, *viaB*. In addition, they demonstrate that the requirement of a functional *viaA* determinant for Vi antigen expression, previously shown in the case of *S. typhi* (5), also holds true for the Vi antigen of *S. paratyphi* C. We would expect that the same relationships demonstrated here between the Vi antigen determinants of *S. typhi* and *S. paratyphi* C should exist also in the Vi-antigen-expressing *Citrobacter* serotypes, as well as in those strains of *S. dublin* in which Vi antigen expression occurs.

In the initial study involving the mapping of the Vi antigen determinants of *S. typhi* (5), the *viaB* genes were shown to occupy a locus to the left (Fig. 1) of the *purA* determinant. The data of the present study point toward a placement to the right of the *mel* determinant for those allelic *S. paratyphi* C and *S. typhi* genes occupying the *viaB* locus. If the distance between the *mel* and *purA* markers is, in fact, as short as indicated in Fig. 1, a cotransduction potential with at least one of these markers would seem to be assured for any genes located between them, even though the transducing fragment carried by P22 may be slightly less than 1% of the total chromosome length (10). Nevertheless, in spite of this seemingly promising situation, not a single transductant expressing the Vi antigen was observed in the present study.

In view of the difficulties observed with regard to the unselected inheritance of the *S. typhi viaB* genes in the conjugation experiments, it might be expected that similar difficulties would be encountered in obtaining their unselected inheritance by transduction. However, no conjugational inheritance problems were observed with the *viaB* genes of *S. paratyphi* C and, with over 70% coinheritance of the *viaB* and *mel* genes in the mating experiments involving that organism, the difficulties encountered in selecting Mel^+ transductants are especially frustrating. In our experience (5), coinheritance percentages of this order in interspecies *Salmonella* matings are indicative of a P22 cotransduction potential for the markers involved. We believe, in the present instance, that, if proximity to the selected marker were the only factor involved, cotransduction of the *S. paratyphi* C and *S. typhi viaB* determinants with *mel* would have been accomplished.

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