RECOMBINATION INVOLVING THE *ilv* CLUSTER OF *SALMONELLA* *TYPHIMURIUM*-*SALMONELLA* MONTEVIDEO HYBRIDS¹

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IN the original report on *Salmonella typhimurium*-*Salmonella montevideo* hybrids (TM hybrids) from this laboratory, it was noted that incorporation of monteideo genic material into the *ilv* (isoleucine-valine) region of the *Salmonella typhimurium* chromosome always involved the four known loci of the *ilv* cluster (GLATZER, LABRIE and ARMSTRONG 1966). The lack of evidence for "partial hybridity," e.g., two loci of typhimurium origin and two of monteideo in the *ilv* region, was explained as a reflection of the close linkage of the *ilv* loci. Many more TM hybrids selected at the *ilv* loci have now been analyzed genetically; and the "all-or-none" incorporation of monteideo material into the *ilv* region has remained the dominant feature of these hybrids. To date, 150 such hybrids have been analyzed (GLATZER, LABRIE and ARMSTRONG 1966; ATKINS and ARMSTRONG 1969; unpublished results); and only nine (6%) display a different pattern of hybridity. Transductional analyses of these latter strains reveal that hybridity has resulted from a very limited incorporation of monteideo material within the locus of selection. Thus, the evidence suggests that, in the production of TM hybrids, incorporation of the entire *ilv* cluster is the favored mode of recombination and that other types of recombination involve insertion of small amounts of DNA.

This report presents two additional lines of evidence that support the supposition that heterologous recombination involving the *ilv* cluster does not result in "partial hybridity" but, rather, in the incorporation of either the whole cluster or a very limited portion of it. These results were obtained from transductional analysis of: 1) recombinants derived from *S. typhimurium* × TM hybrid crosses; and 2) prototrophic hybrids produced by mating *ilv* auxotrophs of *S. montevideo* and *S. typhimurium*.

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MATERIALS AND METHODS

S. typhimurium-*S. montevideo* hybrids were constructed and analyzed as previously described (GLATZER, LABRIE and ARMSTRONG 1966). Techniques utilized in transduction were described by ARMSTRONG and WAGNER (1964).

Bacterial strains: The following *S. typhimurium* strains were used in the study: *ilvC8*, *D18*, and *E16*, each of which shows no spontaneous or chemically-induced reversion to prototrophy (ARMSTRONG and WAGNER 1964); *ilvA118*; *metE338*; and *pdx-4*.

Selection and transductional analysis of recombinants: TM hybrids were used as donors in transduction with *S. typhimurium* *ilv* strains as recipients, and *ilv*⁺ recombinants were selected. To enhance the probability of isolating nonlysogenic recombinants, a multiplicity of infection of one was employed. Recombinants were isolated and tested for lysogeny, and nonlysogenic isolates were analyzed for hybridity by utilizing them as donors in transduction with appropriate auxotrophic strains of *S. typhimurium*.

Production and analysis of TM hybrids derived from *ilv* parental strains: *ilv* strains of *S. montevideo* (ATKINS and ARMSTRONG 1968) were used as male parents in F-mediated conjugation with *ilv* strains of *S. typhimurium*. The F factor was transferred to the *S. montevideo* *ilv* strains from *S. montevideo* SU475 (F⁺ *pur-258*). Because the frequency of prototrophic hybrids obtained in these *ilv* × *ilv* crosses is low, stable *S. typhimurium* auxotrophs were used to minimize the isolation of revertants. Transductional analyses were carried out on the TM hybrids as described in the previous section.

Explanation of terminology: Hybrids are identified according to the following convention: TM-*ilvC71* = seventy-first *S. typhimurium*-*S. montevideo* hybrid isolated by selection at the *ilvC* locus of the *S. typhimurium* parent.

"Percent homology" is the term used to express the recombination observed with respect to a given marker in a cross between a TM hybrid and *S. typhimurium* as a percent of the recombination observed in a homologous *S. typhimurium* cross. A low value of homology (5-25%) implies that the *ilv* locus under investigation is of *S. montevideo* origin in the chromosome of the hybrid; and, conversely, a high value (70% or greater) is recognized as a locus of *S. typhimurium* origin. Intermediate values (those that fall between the low and high values for a given locus) are interpreted to mean that: 1) the locus, although not *montevideo* in origin, is adjacent to a region that is; or 2) a small amount of *S. montevideo* genic material is contained within the locus (usually the locus of selection).

RESULTS

Analysis of recombinants derived from *S. typhimurium* × TM hybrid crosses: Two hybrids, TM-*metE12* and *ilvC18*, were used as donors in transduction with *S. typhimurium* auxotrophs. These two TM strains contain genetic material of *montevideo* origin at the *ilv* cluster and the closely linked *metE* locus (Table 1: TM-*metE12* = 5-15% homology; TM-*ilvC18* = 5-10%). From the *ilvE16* × TM-*metE12* cross, four nonlysogenic prototrophic recombinants (#1-4) were isolated and used as donors in transduction with *S. typhimurium* mutants. It is apparent from the results listed in Table 1 that three of the recombinants (#1-3) were produced as the result of limited incorporation of *montevideo* genetic material into the *ilvE* locus. The 69% value of homology obtained with #1 hints at such an incorporation; however, the results obtained with #2 and #3 show no evidence of *montevideo* material in the *ilv* cluster. This latter observation is interpreted to mean that the incorporation was either too small to be detected by a decreased frequency of recombination or that it involved *montevideo* DNA that was similar in base sequence to the *typhimurium* material it replaced. On

the other hand, the results with #4 clearly show an incorporation of monteideo genetic material involving all *ilv* loci. It should be noted that, because the *metE* locus is not located on the *ilv* transducing fragment, its origin in these recombinants is expected to be typhimurium (115–121%). From the *ilvC8* × TM-*ilvC18* cross, two nonlysogenic recombinants (#1 and 2) were isolated. When these recombinants were analyzed by transduction, they were both found to possess an *ilv* cluster of monteideo origin (Table 1). Recombinant #2 was then used as donor in transduction with *ilvC8*, and a nonlysogenic recombinant (#2a) was isolated from this cross. Upon analysis, #2a was shown to possess an *ilv* cluster of monteideo origin. Thus, the monteideo origin of the *ilv* cluster of TM-*ilvC18* was maintained in two successive heterologous recombinational events.

Although a small number of recombinants were analyzed, only two types of recombination were noted, i.e., very limited amounts of incorporation or the whole *ilv* cluster. These conclusions are very similar to those previously derived from the results of transductional analyses of the 150 hybrids in our collection.

Analysis of prototrophic recombinants produced by mating ilv auxotrophs of S. typhimurium and S. monteideo: Production of TM hybrids was accomplished with the use of *ilv* mutants of the two parental Salmonella species. Prototrophic hybrids were isolated, then analyzed by transduction. The rationale of this experimental design was to enhance the probability of obtaining "partial hybridity" of the *ilv* cluster by ruling out the possibility of incorporation of the entire cluster. This feature of the crosses is illustrated in Figure 1. The arrows in the figure indicate the maximum amount of *ilv* genetic material that can be

TABLE 1
Analysis of TM-metE12, TM-ilvC18, and nonlysogenic recombinants

Donor strains	Percent homology with typhimurium markers					
	120 [†] <u>pdx</u>	<u>ilvE</u>	<u>ilvD</u>	<u>ilvA</u>	<u>ilvC</u>	123 <u>metE</u>
A. TM-metE12	120	7	6	5	4	14
Recombinant # 1 [*]	114	69	90	99	78	121
2	140	110	120	133	132	121
3	96	108	120	115	119	114
4	140	14	19	11	12	115
B. TM-ilvC18	129	10	8	6	5	10
Recombinant # 1 ^{**}	112	17	11	11	14	100
2	96	13	14	16	16	90
2a	130	21	19	22	20	109

[†] Map position, in minutes (SANDERSON 1970).

^{*} Nonlysogenic recombinants (#1–4) obtained from *ilvE16* × TM-*metE12*.

^{**} Nonlysogenic recombinants (#1 and 2) obtained from *ilvC8* × TM-*ilvC18*. Recombinant #2a is a nonlysogenic recombinant derived from #2 × *ilvC8*.

1. *S. typhimurium* *ilvC50* x *S. montevideo* *ilvC706F*⁺ 2. *S. typhimurium* *ilvD18* x *S. montevideo* *ilvC706F*⁺ 3. *S. typhimurium* *ilvC66* x *S. montevideo* *ilvD71IF*⁺

FIGURE 1.—Illustration of the three different crosses carried out with *ilv* mutants of *S. typhimurium* and *S. montevideo*. The arrow under each of the diagrams indicates the maximum amount of *ilv* genetic material that can be expected to be incorporated in the cross.

incorporated in each of the crosses. The following three *ilv* × *ilv* matings were carried out: *ilvC* (typhimurium) × *ilvC* (montevideo); *ilvD* (typhimurium) × *ilvC* (montevideo); and *ilvC* (typhimurium) × *ilvD* (montevideo). Because the linear relationship of the mutation sites in the *ilvC* × *ilvC* cross is not known, production of prototrophic hybrids may or may not involve significant incorporation of montevideo material into the *ilv* cluster (illustration 1 in Figure 1). However, as seen in illustrations 2 and 3, such incorporations can be obtained in the *ilvC* × *ilvD* and the *ilvD* × *ilvC* crosses.

Results of the analyses of twelve hybrids are presented in Table 2. Each of the four strains obtained in the *ilvC* × *ilvC* cross shows a limited amount of incorporation at the *ilvC* locus. Intermediate values of homology are also observed for the *ilvA* and *D* loci as a result of the incorporation at the *ilvC* locus of TM-*ilvC71*. However, as illustrated in Figure 1, hybrid production in this cross may be limited to incorporations that involve only the *ilvC* locus of the cluster. Results

TABLE 2

Transduction analysis of TM hybrids produced from ilv strains of S. typhimurium and S. montevideo

Cross	Hybrid	Percent homology			
		<i>ilvE</i>	<i>ilvD</i>	<i>ilvA</i>	<i>ilvC</i>
<i>S. typh.</i> <i>ilvC50</i> X <i>S. mont.</i> <i>ilvC706</i> F ⁺	TM- <i>ilvC71</i>	105	59	56	51
	TM- <i>ilvC72</i>	111	100	75	99
	TM- <i>ilvC73</i>	86	87	78	83
	TM- <i>ilvC75</i>	104	92	72	97
<i>S. typh.</i> <i>ilvD18</i> X <i>S. mont.</i> <i>ilvC706</i> F ⁺	TM- <i>ilvD72</i>	77	126	111	101
	TM- <i>ilvD74</i>	79	53	50	66
	TM- <i>ilvD75</i>	67	55	42	101
	TM- <i>ilvC81</i>	83	71	61	67
<i>S. typh.</i> <i>ilvC66</i> X <i>S. mont.</i> <i>ilvD711</i> F ⁺	TM- <i>ilvC82</i>	76	82	85	69
	TM- <i>ilvC83</i>	92	97	62	53
	TM- <i>ilvC84</i>	112	122	78	39
	TM- <i>ilvC85</i>	112	68	43	44

obtained with TM-*ilvD72*, 74, and 75 also provide evidence of small incorporations in the region of selection. The patterns of hybridity noted for 74 and 75 are very similar to those previously observed with TM-*ilvD* hybrids that possess limited amounts of montevideo material, i.e., reduced recombination at the *ilvA* locus and, usually, at the *ilvC* locus also (ATKINS and ARMSTRONG 1969). Evidence of limited incorporations is again provided with the results obtained with TM-*ilvC81*–85. As judged by values of homology, the most significant amounts of incorporation have occurred in the production of 84 and 85 (39 and 44% for the *ilvC* locus, respectively). These latter two hybrids were analyzed electrophoretically (ATKINS and ARMSTRONG 1969), and each possesses reductoisomerase activity (product of the *ilvC* gene) that displays the mobility of the typhimurium parent. These results provide no detailed information about the incorporation *per se* (because a single amino acid substitution could account for the difference noted in the electrophoretic mobilities of the typhimurium and montevideo enzymes); however, they do show that the incorporation of montevideo genic material into 84 and 85 did not involve the entire *ilvC* locus. It is apparent from an analysis of the results presented in Table 2 that, when hybrid production is designed to eliminate incorporation of the complete *ilv* cluster of *S. montevideo*, the only recombination observed involves small incorporations.

DISCUSSION

This study on heterologous recombination, involving the *ilv* cluster of *S. typhi*-

murium-*S. montevideo* hybrids, was prompted by the observation that 94% of the TM hybrids that possess hybridity in this region of the chromosome contain genetic material of monteideo origin at all of the *ilv* loci. The remaining 6% contain only limited amounts of incorporation in the immediate region of hybrid selection. These results suggest that, in the production of TM hybrids, incorporation of the entire *ilv* cluster is the most frequent mode of selection. A difference in the order of the *ilv* genes of the parental *Salmonella* species could account for this observation; however, a recent study provides evidence that both species possess the same order (SAWYER and ARMSTRONG 1971). As presented in this report, analysis of six prototrophic recombinants obtained by P22-mediated transduction involving *S. typhimurium ilv* strains and TM hybrids with *ilv* loci of monteideo origin (Table 1) revealed that either incorporations of very limited amounts of genetic material or the complete cluster had occurred. Attempts to construct TM hybrids that possess "partial hybridity" of the *ilv* cluster (by the use of *typhimurium ilv* $\times$ *monteideo ilv* matings) provided evidence that only limited incorporations were involved in the production of such hybrids (Table 2). Thus, data presented in this report support the contention that recombinational events involving the *ilv* cluster in heterologous crosses are not random. It should be noted that in both F-mediated conjugation (production of hybrids) and P22-mediated transduction (prototrophic recombinants analyzed), the patterns of incorporation are the same.

In a report on TM hybrids for the histidine (*his*) loci, ST. PIERRE and DEMEREC (1968) furnished results on three sets of hybrids selected at this region of the *typhimurium* chromosome. One set of hybrids (TM-*hisGO* strains) is comparable to the TM-*ilv* hybrids because incorporation of monteideo genetic material included the entire cluster. The other two sets (TM-*hisF* and -*hisFAHBCD*) displayed intermediate values of homology for the *his* loci. This study on hybridity at the *his* region was complicated by the fact that genes controlling the production of somatic antigens of the cell are located close to the *his* operon. Hence, hybrid production often resulted in strains that were insensitive to bacteriophage P22. Because the hybrids constructed from the *hisGO* parent (possessing a mutated region at *his* loci most distal from the somatic-antigen genes) are the strains that contain a *his* operon of monteideo origin, it is conceivable that the closer proximity of the mutated regions in the *hisF* and *hisFAHBCD* parents had an influence on the types of hybrids that could be studied. These latter hybrids may represent rare types of incorporation that occur at the *his* operon.

The report by DEMEREC and OHTA (1964) on *Salmonella typhimurium*-*Escherichia coli* hybrids was concerned with the *cysC* region. Analysis of two hybrids furnished evidence of "partial hybridity" of this cluster; i.e., the *cysC* and *D* loci were *coli* in origin, and the *cysH*, *I*, and *J* loci were *typhimurium*. These five *cys* loci, however, are located in two distinct groupings with the closely linked *cysC* and *D* loci being separated from the *cysH*, *I*, and *J* loci, likewise closely linked, by a "silent region" that comprises approximately 50% of the total length of the *cysC* region (DEMEREC, GILLESPIE and MIZOBUCHI 1963).

Thus, unlike other gene clusters, the *cysC* region does not possess related genes that are closely linked one to another. Because of the extensive "silent region," it is likely that incorporation of foreign genetic material into one, but not both, of the *cysC* clusters could occur.

The key to the evaluation of hybrid data resides in the interpretation of intermediate values of percent homology. Low and high values of homology require minimal caution in interpretation, but intermediate values (30%–65%) demand close scrutiny. In our studies, an intermediate value of homology for a locus has been explained as the result of 1) a limited incorporation, or 2) typhimurium material adjacent to a region of montevideo origin. DEMEREC and OHTA (1964) interpret intermediate values as coli material adjacent to a region of typhimurium origin. Thus, these latter authors explain intermediate values as a reflection of an *increase* in the frequency of recombination of foreign genetic material, because of the presence of typhimurium material in the transducing fragment, whereas our interpretations emphasize a *decrease* in frequency of recombination because of the presence of foreign genetic material. In this study, the argument can be advanced that "partial hybridity" has been obtained (Table 2: TM-*ilvC71* and 85; and TM-*ilvD74* and 75) but that the evidence for hybridity is masked because of the typhimurium material present in the *ilv* fragment; i.e., intermediate values of homology are obtained instead of low values. It was because of such uncertainties in evaluating intermediate values that the analysis of reductoisomerase activity (product of the *ilvC* gene) by starch gel electrophoresis was developed (ATKINS and ARMSTRONG 1969). As stated in RESULTS, electrophoretic analysis of TM-*ilvC84* and 85 (percent homology = 39% and 44% for the *ilvC* loci, respectively) provided no evidence that the locus is montevideo in origin in these hybrids. These results support the supposition that these intermediate values reflect small incorporations. Although the intermediate values obtained for the *ilvA* and *D* loci cannot be evaluated by a similar technique, it is assumed that they, too, do not represent loci of foreign origin.

The evidence from heterologous crosses that incorporation of the complete *ilv* cluster is favored suggests that maintenance of *ilv* genes of a single genetic origin is occurring in the production of TM hybrids. The studies with bacteriophages T2 and T4 (STAHL and MURRAY 1966) provide evidence that maintenance of clusters of related genes may be critical for the efficient functioning of the gene products within the cellular environment. Transposing this rationale to the *ilv* system in *Salmonella*, it is conceivable that the optimal intracellular conditions for the synthesis of isoleucine and valine require that the enzymes involved be derived from one species, i.e., typhimurium or montevideo in this particular case, and that *ilv* enzymes derived from two species would be detrimental to the production of the amino acids and, hence, to the metabolism of the cell. This idea receives support in the findings by CRONENWETT and WAGNER (1965) that the enzymes of the isoleucine-valine pathway in *S. typhimurium* exist *in vivo* as an enzyme complex associated with the cell membrane. Although this reason for maintenance of clusters is attractive, data to support it have not been obtained. On the contrary, in hybrids produced in *Salmonella* × *Salmonella* matings, gene

(enzyme) substitution involving a number of biosynthetic pathways has no effect on the metabolic efficiency of the hybrid, as judged by growth rate in minimal medium (ARMSTRONG and SPRAGUE 1971). Available evidence, therefore, emphasizes the close relatedness among the species of *Salmonella* that have been used for hybrid production. However, this question about the possible maintenance of clusters should remain open because published data do not offer a definite answer. Hybrid selection, as routinely practiced, may not be yielding the classes of hybrids that would allow for a thorough evaluation of hybridity with regard to gene clusters.

SUMMARY

Evidence accumulated from transductional analysis of *S. typhimurium*-*S. montevideo* hybrids implies that, in heterologous crosses involving the *ilv* cluster, incorporation of all the *S. montevideo ilv* loci is the predominant recombinational event. Data obtained from the analysis of prototrophic recombinants produced from heterologous *ilv* crosses furnish evidence that recombination occurred as an incorporation of the complete *ilv* cluster or as a very limited inclusion of *ilv* genetic material. These results are very similar to those obtained in hybrid production. Attempts to construct hybrids possessing "partial hybridity" of the *ilv* cluster were not successful. These results lend support to the proposal that random incorporations do not occur at any appreciable frequency in heterologous crosses involving the *ilv* cluster of the *Salmonella* chromosome. Although the evidence suggests that a maintenance of an *ilv* cluster of a single genetic origin is occurring in the production of TM hybrids, the biological significance of the phenomenon cannot be established at the present time.

LITERATURE CITED

- ARMSTRONG, F. B. and G. F. SPRAGUE, JR., 1971 *Salmonella* hybrids containing genic material of multiple origins. *Genet. Res.* (in press)
- ARMSTRONG, F. B. and R. P. WAGNER, 1964 Isoleucine-valine requiring strains of *Salmonella typhimurium*. *Genetics* **50**: 957-965.
- ATKINS, C. G. and F. B. ARMSTRONG, 1968 Isoleucine-valine requiring strains of *Salmonella montevideo*. *Microbial Genet. Bull.* **29**: 3. —, 1969 Electrophoretic study of *Salmonella typhimurium*-*Salmonella montevideo* hybrids. *Genetics* **63**: 775-779.
- CRONENWETT, C. S. and R. P. WAGNER, 1965 Over-all synthesis of isoleucine by membrane fractions of *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. U.S.* **54**: 1643-1650.
- DEMEREK, M., D. H. GILLESPIE and K. MIZOBUCHI, 1963 Genetic structure of the *cysC* region of the *Salmonella* genome. *Genetics* **48**: 997-1009.
- DEMEREK, M. and N. OHTA, 1964 Genetic analysis of *Salmonella typhimurium* × *Escherichia coli* hybrids. *Proc. Natl. Acad. Sci. U.S.* **52**: 317-323.
- GLATZER, L., D. A. LABRIE and F. B. ARMSTRONG, 1966 Transduction of *Salmonella typhimurium*-*Salmonella montevideo* hybrids. *Genetics* **54**: 423-432.
- ST. PIERRE, M. L. and M. DEMEREK, 1968 Hybrids of enteric bacteria. I: *S. typhimurium*-*S. montevideo* hybrids for the histidine loci. *Genetics* **59**: 1-9.

- SANDERSON, K. E., 1970 Current linkage map of *Salmonella typhimurium*. Bacteriol. Revs. **34**: 176-193.
- SAWYER, M. E. and F. B. ARMSTRONG, 1971 Order of the *ilv* genes of *Salmonella montevideo*. Mol. Gen. Genetics **109**: 370-372.
- STAHL, F. W. and N. E. MURRAY, 1966 The evolution of gene clusters and genetic circularity in microorganisms. Genetics **53**: 569-576.