ORIENTATION AND ORDER OF LOCI OF THE *met-arg* REGION IN THE *SALMONELLA TYPHIMURIUM* LINKAGE MAP¹

F. B. ARMSTRONG²

*Departments of Biochemistry, Genetics and Microbiology, North Carolina State University,
Raleigh, North Carolina 27607*

Received February 23, 1967

Salmonella typhimurium-*Salmonella montevideo* hybrids were used in P22-mediated transduction to obtain information about the *Salmonella* linkage map (GLATZER, LABRIE and ARMSTRONG 1966). Information on the orientation of the *met-arg* transducing fragment was included. This fragment is located between *metE* and *metA* on the linkage map (SANDERSON and DEMEREC 1965) and contains the loci *metB* and *F*, *asp*, *argA*, *H*, *C*, and *F*. Data on several loci on the fragment were presented. The present study involves a more thorough analysis of the three *typhimurium*-*montevideo* hybrids (TM) that provided information on orientation of the fragment and includes the results of cotransduction tests that were used to determine the order of the loci on the fragment.

MATERIALS AND METHODS

The techniques for using TM hybrids in transductional analysis were presented and discussed by GLATZER, LABRIE and ARMSTRONG (1966). The procedures used for the cotransduction studies can be found in ARMSTRONG and WAGNER (1964). Details concerning the individual experiments are presented with the Tables.

RESULTS AND DISCUSSION

When a hybrid recombinant is used as donor in P22-mediated transduction with auxotrophic *S. typhimurium* strains, the *S. montevideo* portion of the chromosome ("hybrid" or partially "hybrid" transducing fragment) produces fewer transductants than an analogous *S. typhimurium* fragment. Thus, using appropriate *S. typhimurium* auxotrophs as recipients, it is possible to determine the extent of the "hybrid" region in the chromosome of such a recombinant and to place loci on the linkage map.

As seen in Table 1, analysis of three TM hybrid strains shows that the *met-arg* region in each of these strains contains genic material of both *S. typhimurium* and *S. montevideo* origin. The *S. montevideo* region includes the *arg* and *metA* loci but not *metE*, *F* or *B*. The intermediate percentage values obtained with *asp* imply that it is located adjacent to the region containing *S. montevideo* genes (GLATZER, LABRIE and ARMSTRONG 1966), i.e. between the *met* and *arg* loci.

¹ Supported by Public Health Service Research Grant GM 10166-03. Published with the approval of the Director of Research, North Carolina Agricultural Experiment Station.

² Research Career Development Awardee of the Public Health Service (K3-GM-22,647).

TABLE 1

*Analysis by transduction of the met-arg region. Values are expressed as percent homology**

Donor strains	Recipients strains (<i>typhimurium</i>) [†]								
	<i>metE</i>	<i>metB</i>	<i>metF</i>	<i>asp</i>	<i>argH</i>	<i>argC</i>	<i>argF</i>	<i>argA</i>	<i>metA</i>
TM- <i>metA</i> 3‡	92	94	96	53	29	19	25	29	18
TM- <i>metA</i> 4	105	97	119	61	40	31	34	33	14
TM- <i>argA</i> 2	120	87	118	53	28	32	33	30	38

* "Percent homology" is the transduction obtained with a hybrid donor expressed as a percent of that obtained with an *S. typhimurium* donor. Values in the range of 100% indicate that the locus in the hybrid strain is of *S. typhimurium* origin; reduced values indicate genic material of *S. montevideo* origin. For a more detailed presentation of the transduction procedures, see GLATZER, LABRIE and ARMSTRONG (1966).

† Recipient strains used: *metE*338, *metB*218, *metF*185, *asp*-33, *argH*93, *argC*113, *argF*88, *argA*4, and *metA*53.

‡ TM-*metA*3=third *S. typhimurium*-*S. montevideo* hybrid isolated by selection at the *metA* locus of the *S. typhimurium* parent.

These data on the hybrid strains support the following sequence of groups of loci: (*metE*, *metB*, *F*) (*asp*) (*argA*, *C*, *F*, *H*, *metA*), and since the *metB*, *F*, *asp* and *arg* loci are known to be on a single transducing fragment, the order (*metE*) (*metB*, *F*) (*asp*) (*argA*, *C*, *F*, *H*) (*metA*) is established. However, the order of the individual *met* and *arg* loci of the fragment with respect to one another is not shown. Cotransduction was employed to determine this order. This transduction procedure relies on the cotransducibility of the seven loci and on the differences in growth requirements for mutants of these loci. In such two-point crosses the number of donor-type recombinants (representing cotransduction of the donor's alleles of the two loci under analysis) is compared to the number of wild-type recombinants (single transductants). The percentage of wild-type recombinants serves as measure of the recombination frequency between the two loci being tested and is, therefore, an indication of the distance between them, i.e. the lower the frequency of wild-type recombinants the closer the two loci are presumed to be on the linkage map. It should be noted, however, that data such as these need to be interpreted with caution because of the possibility that specific allele effects upon recombination frequencies may be encountered (HARTMAN, LOPER and ŠERMAN 1960).

In tests with *metB* and *F* strains as donors (Table 2), the results suggest that the *metB* locus is more distal from the *asp-arg* region than the *metF* locus and

TABLE 2

Frequency of recombination between met and asp or arg loci

Donor strains	Recipient strains					
	<i>asp</i> -39	<i>asp</i> -33	<i>argA</i> 4	<i>argH</i> 74	<i>argC</i> 113	<i>argF</i> 88
<i>metB</i> 218: Total*	1020	2253	2704	1046	4275	1126
% wild type	85.7	91.3	92.3	92.0	91.8	95.7
<i>metF</i> 185: Total	1138	3245	2824	1117	3444	1319
% wild type	75.5	75.3	84.1	83.9	83.6	89.9

* Total number of recombinants analyzed.

Selection was made for wild- and donor-type recombinants on minimal-medium agar containing 20 µg L-methionine per ml. The transductants were replica plated onto minimal-medium agar to identify the two classes.

TABLE 3

Frequency of recombination between asp and arg loci

Donor strains		Recipient strains						
		1 <i>asp-39</i>	2 <i>asp-11</i>	3 <i>asp-33</i>	4 <i>argF50</i>	5 <i>argF87</i>	6 <i>argA85</i>	7 <i>argA4</i>
<i>argA85</i>	Total†	2311	2052	2774	5405	5428		
	% WT	12.9	13.5	26.8	21.1	49.9		..
<i>argA4</i>	Total	1556	2265	2774	5217	5091		
	% WT	18.1	27.0	34.8	27.7	51.5		
<i>argH74</i>	Total	1482	1426	1623	5737	4679	2300	1888
	% WT	17.5	25.4	37.8	11.0	42.7	14.9	16.9
<i>argH93*</i>	Total	1556	1724	1850	6564	4154	3218	2211
	% WT	14.5	20.9	33.1	14.3	35.1	12.6	15.8
<i>argC113*</i>	Total	1657	1528	2854	5852	3085	2120	2308
	% WT	25.3	25.8	39.3	6.8	25.6	26.5	25.5
<i>argC16</i>	Total	2346	2514	2717	5215	2616	1977	2438
	% WT	30.7	32.1	46.1	5.4	21.2	26.5	25.8
<i>argF88*</i>	Total	1191	1901	2155				
	% WT	29.6	36.5	45.0				
<i>argF87</i>	Total	1253	952	1930				
	% WT	48.4	54.2	56.8				

* Multisite mutant.

† Total number of recombinants analyzed.

Selection was made for wild- (WT) and donor-type recombinants on minimal-medium agar containing appropriate supplements. The transductants were replica plated onto minimal-medium agar to identify the two classes. Supplements (per ml): 20 µg L-arginine with *asp* strains as recipients; 20 µg L-ornithine with *argF* strains as recipients; 30 µg N-acetyl-L-ornithine with *argA* strains as recipients.

imply that the order is: *metB metF asp arg*. Results of reciprocal crosses (not shown) indicate the same arrangement. The order of the four clustered *arg* loci was determined by using *asp*, *argA* and *F* mutants as recipients in crosses with *arg* strains (Table 3). When *asp* strains are used as recipients (Table 3, columns 1, 2, 3), the results show that although *argC* and *F* can be ordered with respect to *asp*, no clear-cut sequence is obtained for *argA* and *H*, and the following order is derived: *asp (argA, H) argC argF*. Tests among the *arg* loci were then used to clarify and supplement these results. Crosses, using *argF* strains as recipients, were carried out with mutants of the other three *arg* loci. The results (Table 3, columns 4, 5) support the placement of *argC* and *F* with respect to *asp* and provide the sequence *argA argH argC argF*. The analogous genes in *Escherichia coli* are arranged in this manner (GLANSDORFF 1965). This order for the *arg* loci is supported by the results obtained when *argA* strains are used as recipients in tests with *argH* and *C* mutants (Table 3, columns 6, 7); the sequence is *argA argH argC*. The multisite mutants have been identified in Table 3 because frequently their mutated regions appear to be closer to the markers being tested than would be expected.

The data in Tables 2 and 3, combined with the results obtained by analysis of the TM hybrids, allow the following sequence to be assigned: *metE metB metF asp argA argH argC argF metA*. HfrA has been shown to transfer *metE* before *metA* and is known to transfer the chromosome in a clockwise fashion, as the

linkage map is generally presented (SANDERSON and DEMEREC 1965). Thus, the loci of the *met-arg* region can be oriented in the linkage map, and it is proposed that the gene sequence, read clockwise from the origin of HfrA, is *metE metB metF asp argA argH argC argF metA*.

SUMMARY

Transductional analysis of *S. typhimurium*-*S. montevideo* hybrids and cotransduction tests provide evidence for the following gene order in the *S. typhimurium* linkage map, read clockwise from the origin of HfrA: *metE met B metF asp argA argH argC argF metA*.

LITERATURE CITED

- ARMSTRONG, F. B., and R. P. WAGNER, 1964 Isoleucine-valine requiring mutants of *Salmonella typhimurium*. *Genetics* **50**: 957-965.
- GLANSDORFF, N., 1965 Topography of cotransducible arginine mutations in *Escherichia coli* K-12. *Genetics* **51**: 167-179.
- GLATZER, L., D. A. LABRIE, and F. B. ARMSTRONG, 1966 Transduction of *Salmonella typhimurium*-*Salmonella montevideo* hybrids. *Genetics* **54**: 423-432.
- HARTMAN, P. E., J. C. LOPER, and D. ŠERMAN, 1960 Fine structure mapping by complete transduction between histidine requiring *Salmonella* mutants. *J. Gen. Microbiol.* **22**: 323-353.
- SANDERSON, K. E., and M. DEMEREC, 1965 The linkage map of *Salmonella typhimurium*. *Genetics* **51**: 897-913.