ORIENTATION OF THE ISOLEUCINE-VALINE GENES IN THE *SALMONELLA TYPHIMURIUM* LINKAGE MAP[1]

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Received January 18, 1966

SOME operons in *Escherichia coli* and *Salmonella typhimurium* are oriented in a clockwise direction while others are oriented in a counterclockwise direction in the respective linkage maps as usually drawn (SANDERSON 1965). Knowledge concerning the orientation of operons is important because of its bearing on models of gene transcription and regulation.

A cluster of at least four genes is involved in the biosynthesis of isoleucine and valine in Salmonella. The genes in this cluster have been ordered with respect to one another, but the sequence has not previously been oriented with regard to other loci on the *S. typhimurium* linkage map (ARMSTRONG and WAGNER 1964; GLANVILLE and DEMEREC 1960). The present communication presents evidence on the orientation of this gene cluster; the orientation is the same as reported for an analogous gene cluster in *E. coli* (RAMAKRISHNAN and ADELBERG 1965).

MATERIALS AND METHODS

Bacterial strains were obtained from the collections of M. DEMEREC, Brookhaven National Laboratory and P. E. HARTMAN, Johns Hopkins University. All mutants were derived from strain LT-2 except the histidine regulatory mutants (*hisR*) which are derivatives of strain LT-7. Phage P22 was used for the transductional crosses. Phage stocks were prepared and stored by the method of HARTMAN (1956). Crosses were performed by mixing phage and bacteria directly on the selection medium at a multiplicity of infection of 10. Plates were scored after 48 hours. Conjugation crosses were performed by the method of SANDERSON and DEMEREC (1965).

RESULTS

The *hisR* gene of Salmonella is involved in regulation of the levels of histidine biosynthetic enzymes and is located between the cluster of genes controlling isoleucine-valine biosynthesis (*ileA* and *ilv* genes) and a gene involved in methionine biosynthesis (*metE*). Mutations in the *hisR* gene are weakly cotransducible with each of these loci in P22-mediated transduction tests (ROTH and

[1] A portion of the research was carried out at the Brookhaven National Laboratory under auspices of the U.S. Atomic Energy Commission; the remainder was supported by research grant AI-01650, National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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TABLE 1

Results of two-point transduction tests

Donors	Recipients							
	ilvC401		*ilvC13*		*ilvA8*		*ilvA14*	
	Colonies scored	%R	Colonies scored	%R	Colonies scored	%R	Colonies scored	%R
hisR1200	2072	0.8	878	0.9	478	5.9	1183	1.9
hisR1201	3300	0.6	1221	1.7	422	5.2	1624	1.5
hisR1203	2031	0.5	606	1.0	1023	2.2	1039	0.9
hisR1204	2684	1.0	. . .	. .	553	3.6	. . .	. .
hisR1205	1551	0.6	805	0.6	285	4.6	408	2.0
hisR1208	989	0.6	648	1.4	13426	5.9	656	2.3
Totals for all *hisR*	12637	0.7	4158	1.2	16187	5.4	4910	1.6
	Pooled results for *ilvC*				Pooled results for *ilvA*			
Totals for all *hisR*	16705 scored		0.81%R		21097 scored		4.5%R	

P22 phage grown on various *hisR* mutants was used to transduce *ilv* mutant cells (require isoleucine plus valine) to prototrophy. Since the *hisR* mutation causes formation of a rough (R) colony on the 2% glucose medium used, recombinants in which the donor *hisR* mutation had been cotransduced with *ilv*$^+$ were recognized by their colony morphology. Percent cotransduction (%R) of various *hisR* mutations with several *ilv* mutations are presented.

HARTMAN 1965). Two-point transduction tests (Table 1) indicate that mutations in *hisR* are more closely linked with each of two mutations in the *ilvA* gene than with each of two mutations in the *ilvC* gene. Since the gene order within the *ilv* cluster is established as *ilvC ilvB ileA ilvA* (ARMSTRONG and WAGNER 1964; GLANVILLE and DEMEREC 1960), our results indicate that this ordering can be extended to *ilvC ilvB ileA ilvA hisR metE*.

This map order is verified by three-point tests. In these crosses, one parent is an *ilv* mutant requiring both isoleucine and valine; the other parent carries two markers in the region of interest, an *ileA* mutation which leads to a requirement for isoleucine alone, and a *hisR* mutation which causes a rough colony phenotype on media containing 2% glucose (HARTMAN, ROTH, and AMES 1964). The two strains used, *hisR1208 ileA151* and *hisR1203 ileA152*, also carry an unlinked histidine mutation (*hisH107*) which does not enter into any of the crosses reported here. The *ileA* mutations are diethylsulfate-induced in the *hisR hisH* double mutant. The newly introduced mutations are assigned to the *ileA* locus on the basis of a requirement for isoleucine alone. Enzyme assays performed by DR. FRANK B. ARMSTRONG (personal communication) confirm this assignment.

Figure 1 presents the results and interpretation of each of the three-point tests. In Cross 1, isoleucine-valine independent recombinants are selected and scored directly on histidine-supplemented medium for the presence (rough colony) or absence (smooth colony) of the unselected *hisR* mutation. In the reciprocal transduction tests (Crosses 2 and 3a), all *ilv*$^+$ recombinants are examined, regardless of whether they are *ile*$^+$ or *ile*. The *ile* class grows on medium lacking isoleucine due to the "feeding" of *ileA151* by the large excess of nontransduced *ilvA8* cells present on the transduction plate. In Cross 3b, the recombinants are selected on medium containing isoleucine (as in Cross 3a) and then tested for the ability to

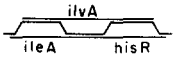
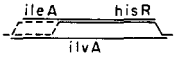
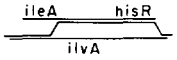
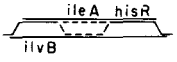
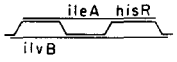
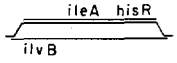
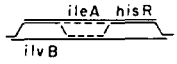
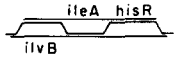
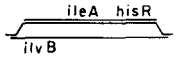
CROSS	RECIPIENT	DONOR	ADDITIONS TO MINIMAL MEDIUM*	COLONY SIZE	SELECTED	TOTAL COLONIES SCORED	PERCENT DONOR hisR GENE	INTERPRETATION**
1.	hisRI208 ileAI51	ilvA8	0	Large	ilv^+ile^+	2,408	0.95	
2.	ilvA8	hisRI208 ileAI51	0	Large	ilv^+	7,657	4.9	
3a.	ilvA8	hisRI208 ileAI51	ile	Large	ilv^+	2,910	5.5	
3b.	ilvA8	hisRI208 ileAI51	ile[x] 0	Large	ilv^+ile^+	410	3.6	
4.	ilvB10	hisRI208 ileAI51	ile	Large	ilv^+	2,970	1.9	
5.	ilvB10	hisRI208 ileAI51	0	Large	ilv^+ile^+	971	0.1	
				Small	ilv^+ile^-	4,264	1.6	
6.	ilvB10	hisRI203 ileAI52	ile	Large	ilv^+	2,299	0.70	
7.	ilvB10	hisRI203 ileAI52	0	Large	ilv^+ile^+	623	<0.16	
				Small	ilv^+ile^-	3,746	0.35	

FIGURE 1.—Results and interpretation of three-point transduction tests.

* Medium for each cross contained 2% glucose to permit scoring of rough colony phenotype of *hisR* mutation. Whenever a double mutant in the region of interest was used as a recipient, 50 μg/ml histidine was added to the medium since these strains also carry an unlinked *hisH* mutation.

** Upper line represents transducing fragment and lower line represents chromosome of recipient bacterium. Positions of crossovers are depicted which give recombinants that carry the *hisR* gene of the donor. Dashed lines indicate alternate crossover possibilities.

[x] Plated on minimal medium containing isoleucine and then replica plated onto medium lacking isoleucine for examination of the ilv^+ ile^+ class of recombinants.

grow in the absence of *ilvA8* cells on medium lacking isoleucine. This allows scoring of the colony phenotype (rough or smooth) among the prototrophic ilv^+ ile^+ class alone. Comparison of the data for Crosses 2 and 3 demonstrates that the presence of isoleucine in the plating medium does not influence significantly the linkage between *ilv* and *hisR*. From Crosses 1 to 3, the gene order *ileA ilvA hisR* is inferred since the latter two crosses yield greater frequencies of the unselected *hisR* marker (through double crossovers) than the former cross (through quadruple crossovers). Similar reasoning has been applied to the results of Crosses 4 and 5 and to Crosses 6 and 7. In these cases, a lesser degree of feeding by the background of recipient bacteria allows direct scoring of the ile^+ and *ile* (smaller colony) recombinants on the isoleucine-free plating medium. From Crosses 4 to 7 the gene order *ilvB ileA hisR* is inferred.

The results of the transduction tests allow placement of the genes in the order

ilvC ilvB ileA ilvA hisR metE but fail to reveal their orientation in the linkage map. However, the sequence is oriented by results of interrupted conjugation crosses, carried out by methods previously described (SANDERSON and DEMEREC 1965), between donor strain SR305 (HfrA *hisD23 gal-50 str*^{s}) and strain SB109 (*metE338 ilvC401 str*^{r}) as recipient. (*gal* = galactose-negative; *str*^{s} and *str*^{r} = streptomycin sensitive and resistant, respectively.) Selection against donor bacteria is achieved by inclusion of 1200 μg/ml streptomycin and by the absence of histidine from the plating medium. Separate selections among recipient bacteria are made for *ilv*$^+$ (methionine present in the medium) and *met*$^+$ (isoleucine and valine present in the medium) recombinants.

Figure 2 shows the times of entry of the *ilvC* and *metE* loci; the *ilvC* locus appears to enter at about 6 to 7 minutes, the *metE* locus about one minute later. This conclusion is verified by analysis of the recombinants for unselected markers (Table 2). When *ilvC*$^+$ is the selected recombinant class, the frequency of the unselected *metE*$^+$ marker among the recombinants increases with increased mating time. This indicates that *metE* is transferred after *ilvC*. On the other hand, when the *metE*$^+$ class of recombinants is selected, the frequency of recombinants also carrying *ilvC*$^+$ does not change with time but remains constant at approximately 85%. These results indicate that *ilvC* precedes *metE* since additional transfer time does not increase the frequency of its incorporation among the *metE*$^+$ recombinants. HfrA is known to transfer the chromosome clockwise as the map is usually drawn (SANDERSON and DEMEREC 1965).

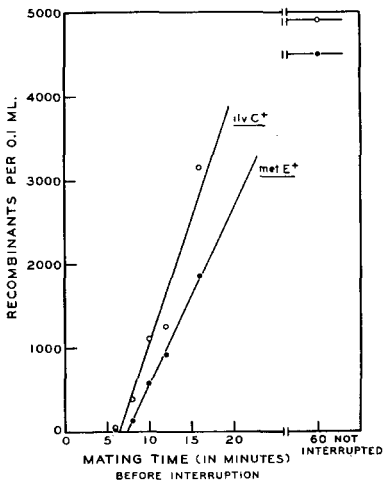


FIGURE 2.—Time of entry of *metE* and *ilvC* loci. SR305 (HfrA) was mated with SB109 and transfer was interrupted at various times. Selections were made for *metE*+ and for *ilvC*+ at each interruption time and the number of each type found per 0.1 ml of the mating mixture (4×10^5 donor cells) is presented. *ilvC*+ recombinants (open circles) appear after 6 to 7 minutes of mating; *metE*+ recombinants (filled circles) appear approximately one minute later.

TABLE 2

Analysis of recombinants from conjugation cross

Marker selected	Mating time (minutes)	Number of tested recombinants carrying unselected marker			Percent of recombinants carrying unselected donor marker
		ilv+ *met*+	*ilv*+ *met*	*ilv met*+	
ilvC+	6	0	6	..	0
	8	16	89	..	15
	10	39	79	..	33
	12	10	22	..	31
	16	53	29	..	65
	60	43	16	..	73
	(Not interrupted)				
metE+	8	9	..	2	88
	10	43	..	4	85
	12	99	..	24	81
	16	68	..	13	84
	60	96	..	12	89
	(Not interrupted)				

SR305 (HfrA *hisD23 gal-50 str*^{s}) was crossed with SB109 (*metE338 ilvC401 str*^{r}) and the donor strain was counter-selected with 1200 μg/ml streptomycin and lack of histidine in the selection medium.
When *ilvC*+ recombinants were selected, *metE*+ served as the unselected marker and when *metE*+ was the selected recombinant class, then *ilvC*+ was the unselected marker.

DISCUSSION

Together with the gene order derived from the results of the transduction experiments, the results of the conjugation experiment allow us to propose that the order of loci, reading clockwise from the origin of HfrA, is: *ilvC ilvB ileA ilvA hisR metE*. Nomenclature of the genetic loci involved is not uniform for *S. typhimurium* and *E. coli*. Therefore the orientation found for the *ilv* cluster of the two organisms can only be compared by considering the enzymes controlled by these loci. The enzymes, their position in the pathway and the genetic loci controlling them are presented in Table 3. The genetic loci are in order reading clockwise around the linkage map as it is generally drawn. Thus the orientation of loci in *S. typhimurium* is the same as that reported for *E. coli* (RAMAKRISHNAN and ADELBERG 1965). If the positions of the *ilv* operators are the same in *S. typhimurium* as they are in *E. coli* (RAMAKRISHNAN and ADELBERG 1965), then

TABLE 3

Genetic loci of S. typhimurium *and* E. coli *controlling isoleucine-valine biosynthetic enzymes*

Enzyme	Transaminase	Dehydrase	L-Threonine deaminase	Reducto-isomerase	Condensing enzyme
Position in pathway	5	4	1	3	2
Genetic locus symbol in *S. typhimurium*	*ilvC*	*ilvB*	*ileA*	*ilvA*	....
Genetic locus symbol in *E. coli*	*ilvE*	*ilvD*	*ilvA*	*ilvC*	*ilvB*

(ADELBERG + RAMAKRISHNAN 1965; ARMSTRONG and WAGNER 1964.)

the *ilv* operons, like the leucine operon, are polarized counter-clockwise in the *S. typhimurium* linkage map; the tryptophan and histidine operons are polarized in a clockwise direction (SANDERSON 1965).

SUMMARY

The cluster of genes involved in isoleucine-valine biosynthesis has been oriented with respect to *hisR* by means of P22-mediated two- and three-point transduction crosses. The *hisR* gene lies between the *ilv* cluster and the *metE* locus. Interrupted conjugation crosses show that HfrA transfers *ilvC* before *metE*. Since HfrA transfers the bacterial chromosome in a clockwise order, we infer that the gene sequence in this region, reading clockwise from the origin of HfrA, is: *ilvC ilvB ileA ilvA hisR metE*.

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