Genetic Analysis of *fla* and *mot* Cistrons Closely Linked to *H1* in *Salmonella abortusequi* and its Derivatives

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(Accepted for publication 29 September 1971)

SUMMARY

Spontaneous non-flagellate and paralysed mutants were isolated from a phase-I stable strain of *Salmonella abortusequi* and its *H1*-replaced derivatives. Trail production in transduction between these mutants resulting from complementation was tested. Fifteen cistrons were recognized: *flaA*, *flaB*, *flaC*, *flaD*, *flaE*, *flaF*, *flaK*, *flaL*, *flaM*, *motA*, *motB*, and *H1* (*ah₁*) which were already known, and three new ones, *flaN*, *flaP*, and *flaQ*. All, except *flaF* and *flaM*, were co-transducible with *H1*. *FlaA* mutants fell into three subgroups, *flaAI*, *flaAII*, and *flaAIII*. Some paralysed mutants belonged to the same group as non-flagellate *flaAII* mutants, and their average numbers of flagella per bacterium varied with the strain from 0 to nearly the same as wild-type. Several single site *flaAII* and *flaN* mutants showed non-reciprocal complementation; as recipients in complementation tests they gave shorter and often fewer trails than when they were donors. Deletion mutants covering most or all of the *flaAII* or *N* cistrons showed normal complementation, suggesting the shorter trails with single site mutants to be the result of incomplete complementation by defective products of the mutated gene.

Fla and *mot* mutations closely linked to *H1* were marked with deletion mutants and their sequences determined in three-factor reciprocal crosses. Two gene clusters were found, and the sequence of cistrons in these clusters was *-flaD-flaB-flaQ-flaP-flaN-flaAIII-flaAII-flaAI-H1(ah₁)-flaL-* and *-(flaE,flaK)-motA-motB-flaC-flaM-*. All the paralysed *flaAII* mutations were located at one end of *flaAII*.

INTRODUCTION

In *Salmonella*, various genes controlling the formation and function of flagella are known. *H1* and *H2* are the structural genes for the flagellar protein flagellin, in phases 1 and 2. They are not co-transducible: *H1* is located between *his* and *tre*, and *H2* close to *purC* (Smith & Stocker, 1962; Mäkelä, 1964; Enomoto, 1966*b*; summarized in Sanderson, 1970). *Ah₁* and *ah₂*, closely linked to *H1* and *H2* respectively, are the genes determining activity of their adjacent flagellin structural genes (Iino, 1961, 1962). Another group of genes (*fla*) play a role in the production of flagella other than in the determination of the structure of flagellin. Ten *fla* cistrons have been recognized in complementation tests, that is, *flaA*, *B*, *C*, *D*, *E*, *F*, *G*, *J*, *K* and *L*. Except for *flaF* and *flaG*, they are co-transduced with *H1* by P22 phage (Joys & Stocker, 1965; Iino & Enomoto, 1966; Yamaguchi & Iino, 1969). *FlaF* was located

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Fig. 1. General linkage map of *Salmonella* showing the location of *fla* and *mot* genes. (After Sanderson, 1970.)

between the *gal* and *trp* operons by colicin mediated conjugation (Smith & Stocker, 1962). The position of *flaG* is unknown. Recently an additional *fla* cistron, *flaM*, was found which is near *Hr* but is not co-transduced with it (P. Vary & B. A. D. Stocker, personal communication). Genes designated *mot* regulate motility. The *mot* mutants are paralysed, that is they are non-motile though normally flagellated. Three *mot* cistrons, *motA*, *B* and *C*, have been reported, all of which are co-transducible with *Hr* (Enomoto, 1966*a, b*). A general linkage map of *Salmonella* is shown in Fig. 1.

Thus, most of the genes concerning formation and function of flagella are closely linked to *Hr* locus. The relative positions on the chromosome of different groups of these genes have been reported (Table 1); a complete and comprehensive genetic map of these genes could provide fundamental information on the genetic control of the formation and function of flagella. In this study non-motile mutants were isolated from a phase 1 stable strain of *Salmonella abortusequi* and its *Hr*-replaced derivatives, they were grouped in complementation tests, and mapped using appropriate deletion mutants.

Table 1. Previous reports on the sequence of *fla* and *mot* cistrons closely linked to *Hr* in *Salmonella*

Sequence	Species	Method*	Reference
- <i>flaJ-flaB-flaD-flaC-flaA-Hr</i> -	<i>Salmonella typhimurium</i>	1	Iino & Enomoto (1966)
- <i>his-motC-Hr-motB-motA-leu</i> -	<i>S. typhimurium</i>	2	Enomoto (1966 <i>b</i>)
- <i>motC-Hr-flaK-flaC</i> -	<i>S. typhimurium</i>	3	Enomoto (1967)
- <i>flaJ-flaB-flaD-flaC-flaA-motC-Hr</i> -			Joys (1968)
-(<i>flaB, flaD</i>)- <i>flaA-Hr-flaL</i> -	<i>S. abortus-equi</i>	3	Yamaguchi & Iino (1969)
- <i>flaB-flaD-flaA-Hr</i> -	<i>S. typhimurium</i>	3	Joys & Stocker (1969)
- <i>his-supW-flaD-flaB-flaA</i> -	<i>S. typhimurium</i>	3	D. B. Fankhauser, personal communication

* 1 = co-transduction frequency with *Hr*; 2 = sexual recombination; 3 = three-factor cross.

METHODS

Bacteria. Non-motile mutants were isolated from stable phase 1 derivatives SJ241, TR6, TR16, and TR17 of *Salmonella abortusequi* strain SL23 (Table 2*a*). The origin and some characteristics of non-motile mutants used as representatives of established cistrons appear in Table 2*(b)*.

Media. Nutrient broth comprised 1 % (w/v) peptone and 1 % (w/v) meat extract, adjusted to pH 7.2, to which 1.5 % (w/v) agar was added to give nutrient agar. Semisolid medium used in transduction experiments contained 8 % (w/v) gelatin and 0.28 % (w/v) agar in nutrient broth, and that used to test leaky motility was similar but contained 0.1 % (w/v) agar. All cultures were incubated at 37°.

Isolation and characterization of non-motile mutants. The procedure was similar to that of Enomoto & Iino (1963), except that phage M8 instead of χ was used as the selective agent. Phage χ attacks motile *Salmonella* organisms other than those with *g*-complex flagellar antigens, but cannot attack non-motile bacteria (Meynell, 1961). Phage M8 is a host-range mutant of χ able to attack motile salmonellas with *g*-complex antigens (Sasaki, 1962; Yamaguchi, 1968). M8 was added at a multiplicity of about 1 to a broth culture containing about 10^8 bacteria/ml of an actively motile parent strain. After overnight incubation, the culture was streaked on to semi-solid medium to give well isolated colonies. Non-motile mutants formed compact colonies, whereas motile bacteria not attacked by the phage produced swarms. Non-flagellate and paralysed mutants were first distinguished from each other by their colony morphology: the former gave large pale colonies and the latter small dense colonies (Enomoto & Iino, 1963). More accurate distinction between the two types of mutants was obtained by slide-agglutination with anti-flagella sera. Reversion and leakiness were tested by streaking overnight broth cultures of mutants on semisolid medium plates and examining them for swarms and trails after 48 h incubation.

Transduction. P22 phage lysates were prepared by the soft-agar layer method (Adams, 1959), titrated on strain SJ241, and diluted in broth to give suspensions containing 1×10^{10} infective units/ml.

To determine if two non-motile mutation sites overlap each other, the same volume of an overnight broth culture of the recipient (about 1×10^8 bacteria/ml) and the donor phage lysate were mixed to give a multiplicity of infection of about 5, and an 0.05 ml sample of the mixture from a 0.1 ml syringe brushed in four to five lines on each of five sets of plates (9 cm diam.) of semisolid medium. After 20 h incubation the plates were examined for swarms resulting from recombination between the two mutants. When no swarm was produced on any of the five plates, the two mutations were judged to overlap. In control experiments, in which SJ241 (wild-type) was used as donor and non-motile mutants as recipients, 1000-fold dilutions of the transduction mixtures gave more than one swarm/five plates. A yield of less than 10^{-3} motile recombinants of those in the control cross could therefore be detected.

To measure co-transduction frequency with *Hr*, the above method was used except that the wild-type strain SJ241 (*Hr-a*) or TR6 (*Hr-gt*) was used as donor. Appropriate dilutions of the transduction mixture to give isolated swarms were brushed in line on semisolid medium. After incubation for 20 h, swarms were isolated on nutrient agar and after overnight incubation their flagellar antigens determined by slide-agglutination. When the co-transduction frequency was very low, tenfold serial dilutions of the transduction mixture were brushed on both semisolid medium without serum and with enough antiserum specific for the recipient-type flagellar antigen to prevent the spreading growth of homologous type

swarms. Swarms appearing on the latter medium were regarded as transductants having received *Hr* together with the wild-type allele of the mutant gene from the donor.

In complementation tests, a P22 donor phage lysate was mixed with a broth culture (1×10^8 bacteria/ml) of a recipient non-motile mutant at a multiplicity of 1.0. This mixture

Table 2. *Bacteria*

(a) Strains used for the isolation of non-motile mutants

Strain no.	Flagellar antigen		Derivation
	Phase 1	Phase 2	
SJ241	<i>a</i>	(<i>enx</i>)*	Phase-1 stable strain derived from <i>Salmonella abortusequi</i> SL23
TR6 (= SJ925)	<i>gt</i>	(<i>enx</i>)	Derivative of SJ241 with <i>Hr-gt</i> transduced from <i>Salmonella</i> sp. SJ6
TR16 (= SJ929)	<i>fg</i>	(<i>enx</i>)	Derivative of SJ241 with <i>Hr-fg</i> transduced from <i>S. derby</i> NCTC1729
TR17 (= SJ930)	<i>gm</i>	(<i>enx</i>)	Derivative of SJ241 with <i>Hr-gm</i> transduced from <i>S. enteritidis</i> NCTC4196

* () = unexpressed antigen type.

(b) Non-motile strains used as standards in complementation tests

Complementation group	Mutant no.	Strain no.	Reversion*	Parent strain†	Reference‡
<i>flaA</i>	22	SL481	—	TM2	3
	23	SL482	+	TM2	3
	34	SJ35	+	TM2	3
	37	SJ80	+	TM2	3
	41	SJ373	—	TM2	3
	55	SL830	—	M11	4
	56	SL678	—	M10	4
	57	SL829	—	M10	4
	61	SL826	—	M6	4
	66	SL694	+	SD14	4
<i>flaB</i>	67	SL695	+	SD14	4
	50	SL821	—	M5	4
	59	SL822	+	M5	4
<i>flaC</i>	21	SL480	—	TM2	3
	31	SJ31	+	TM2	3
	38	SJ81	+	TM2	3
<i>flaD</i>	42	SJ374	—	TM2	3
	58	SL830	—	M12	4
<i>flaE</i>	60	SL825	+	M6	4
<i>flaF</i>	25	SL488	—	TM2	3
<i>flaJ</i> (= <i>flaE</i>)	28	SW1154	+	TM2	3
<i>flaK</i>	44	SJ400	—	TM2	3
<i>flaL</i>	204	SJ1585	—	TR16	6
<i>flaM</i>	682	SL4062	+	TM2	5
	699	SL4063	+	TM2	5
<i>Hr</i> or <i>ah₁</i>	<i>ah₁-I</i>	SW1061	+	TM2	2
<i>motA</i>	257	SJ476	+	TM2	1
<i>motB</i>	275	SJ603	—	TM2	1
<i>motC</i>	244	SJ448	—	TM2	1
	272	SJ600	—	TM2	1
	279	SJ608	—	TM2	1

* + = reversion; — = no reversion.

† TM2, a strain of *Salmonella typhimurium*; M5, M6, M10, M11, and M12, derivatives of TM2 with mutant forms of phase 1 antigen *i*; SD14, a derivative of TM2 with *Hr-b* and *H2-enx* transduced from *S. abony* strain sw803.

‡ 1 = Enomoto (1966a); 2 = Iino (1961); 3 = Iino & Enomoto (1966); 4 = Joys & Stocker (1965); 5 = P. Vary & B. A. D. Stocker, personal communication; 6 = Yamaguchi & Iino (1969).

was then diluted tenfold into broth and a known volume of the dilution brushed in line on plates of semisolid medium. After 20 h incubation, the formation of trails was scored using a binocular microscope ($\times 15$ magnification). Trail formation in the transduction of motility represents the transmission of a non-replicated extrachromosomal fragment carrying the donor functional allele for motility through many generations to one daughter cell (heterogenote) at each cell division making it motile (Lederberg, 1956; Stocker, 1956). This only happens if complementation occurs between donor and recipient alleles.

Antisera. Both anti-*a* and anti-*gt* flagella sera were used. Overnight broth cultures of SJ241 (*a*) and TR6 (*gt*) were centrifuged and the pellets resuspended in 0.5 % formalinized saline to give about 1×10^8 bacteria/ml. Rabbits were immunized by one intraperitoneal and five successive intravenous injections with a total of 6 ml of the bacterial suspension at three-day intervals, and bled one week after the final injection. The antisera were processed according to Edwards & Ewing (1955). Antibodies specific to the somatic antigens were removed by absorbing the sera with a non-flagellate bacterial strain derived from SJ241.

Observation of flagella. Number and length of flagella of paralysed mutants were examined by the method of Leifson (1951). Four hundred bacteria of each strain were examined.

RESULTS

Characteristics of the mutants. The spontaneous non-motile mutants isolated from SJ241, and its *H1*-replaced derivatives were classified into two groups by reciprocal transduction; single site mutants whose sites of mutation do not overlap, and deletion mutants which cover two or more single site mutations. The characteristics of the mutants used in subsequent experiments are recorded in Table 3. Of the 90 single site mutants, 31 occasionally produced motile revertants. Eleven of them were leaky in that they yielded spontaneous trails in the semisolid medium in a leakiness test.

Complementation grouping of non-motile mutants. Complementation tests were carried out by transduction between pairs of single site mutants. Non-motile strains used as representatives of known cistrons were used only as donors. The numbers of trails were scored for each combination and the degree of complementation expressed according to the criteria of Iino & Enomoto (1966). 0 indicates the production of no trails, 1, 2 and 3 indicate trail production at less than 10 %, between 10 and 50 %, and more than 50 % of the controls, respectively. Control transductions in which mutants with defects in different cistrons were used as recipients gave more than 500 trails. Mutants were assigned to different groups if the figure for trail formation was 3 and to the same group if it was less. On this basis 15 complementation groups were recognized and the results appear in Tables 3 and 4. Eleven groups corresponded well to the known *fla* and *mot* cistrons, *flaA*, *B*, *C*, *D*, *E*, *F*, *K*, *L*, and *M*, and *motA* and *B*. *FlaF* mutants known to be located far from *H1* on the chromosome were omitted from this study.

Iino & Enomoto (1966) reported that *Salmonella typhimurium flaA* mutants fell into three subgroups, such that every cross involving a pair of mutants from different subgroups gave a trail rating of 2 or more: one of the subgroups comprised *flaA37*, another *flaA23* and *34*, and another *flaA22*. Among the new mutants those not complementing *S. typhimurium* mutant *flaA41* also fell into three subgroups, designated *flaAI*, *AII* and *AIII*, respectively (Table 4a). It should be noted that nine paralysed mutants belonged to *flaAII*. Except for the combinations showing non-reciprocal complementation described below, mutants of *flaAI* complemented well, and were well complemented by, all the mutants of the other subgroups, but in some combinations between the *flaAII* and the *flaAIII* mutants trails were shorter

Table 3. *Characteristics of non-motile mutants isolated*

Hr alleles are shown in parentheses. Mutants with *Hr-a* were derived from SJ241, with *Hr-gt* from TR6 with *Hr-fg* from TR16, and with *Hr-gm* from TR17.

		(a) Single site mutants		
		Mutant no.		
Comple- mentation group		Stable	Revertible	
			Not leaky	Leaky
<i>flaAI</i>		1310(a), 1344(a)	—	—
<i>flaAII</i>		297(gm), 1223(a), 1264(a), 1281(a), 1296(a), 1297(a), 1299(a), 1305(a), 1322(a), 310(a),* 1259(a),* 1439(a),* 1441(a),* 1446(a)*	1241(a), 1255(a), 1288(a), 1247(a),* 1440(a),* 1442(a),* 1445(a)*	—
<i>flaAIII</i>		327(a), 332(a), 1315(a)	1251(a), 1341(a)	1329(a), 1343(a), 220(fg)
<i>flaB</i>		—	1272(a), 1351(a)	1218(a), 1250(a), 1256(a)
<i>flaC</i>		1141(gt), 1142(gt), 1143(gt), 1144(gt)	1146(gt)	1145(gt)
<i>flaD</i>		211(fg), 1227(a)	—	1353(a)
<i>flaE</i>		1152(gt), 1162(gt), 1171(gt)	1186(gt)	—
<i>flaF</i>		1164(gt), 1173(gt)	1168(gt), 1179(gt)	—
<i>flaK</i>		1159(gt), 1194(gt), 1195(gt)	—	1190(gt)
<i>flaL</i>		263(gm), 309(a), 351(gt), 1411(a), 1413(a), 1415(a), 1598(gt), 1599(gt), 1600(gt)	—	—
<i>flaM</i>		1467(gt)	—	—
<i>flaN</i>		1148(gt), 1201(a), 1291(a)	1225(a)	—
<i>flaP</i>		—	—	1337(a)
<i>flaQ</i>		1202(a), 1205(a), 1235(a), 1246(a), 1309(a)	1324(a), 1331(a)	1147(gt)
<i>Hr (ah₁)</i>		aM1(a), aM2(a), aM3(a), aM5(a), aM6(a), aM7(a)	—	—
<i>motA</i>		811(gt)	815(gt)	—
<i>motB</i>		816(gt)	810(gt)	—

* Mutants paralysed but belonging to *flaAII*.

(b) Deletion mutants			
Mutant no.	Complementation group	Mutant no.	Complementation group
204(fg)	<i>flaL</i>	1234(a)	<i>flaAIII</i>
221(fg)	<i>flaB, D</i>	1238(a)	<i>flaAI, AII, AIII</i>
227(fg)	<i>flaAI, AII</i>	1245(a)	<i>flaB</i>
234(fg)	<i>flaC, M</i>	1270(a)	<i>flaAI, AII, AIII, B, N, P, Q</i>
286(gm)	<i>flaAII</i>	1293(a)	<i>flaN, P</i>
291(gm)	<i>flaE, K, C, motA, B</i>	1319(a)	<i>flaAI, AII, AIII, L, Hr(ah₁)</i>
302(a)	<i>flaE, K, motA, B</i>	1328(a)	<i>flaAIII</i>
306(a)	<i>flaAII</i>	1330(a)	<i>flaAII</i>
316(a)	<i>flaB, D</i>	1336(a)	<i>flaAII</i>
373(gt)	<i>flaAI, AII, AIII, B, D, N, P, Q</i>	1346(a)	<i>flaB, D, N, P, Q</i>
375(gt)	<i>flaB, D, N, P, Q</i>	1350(a)	<i>flaB, D</i>
1131(a)	<i>flaK</i>	1383(a)	<i>flaAI, AII</i>
1139(gt)	<i>flaE, K, motA</i>	1423(a)	<i>flaL Hr(ah₁)</i>
1140(gt)	<i>flaC, M</i>	1426(a)	<i>flaL Hr(ah₁)</i>
1149(gt)	<i>flaC</i>	1594(gt)	<i>flaB, D</i>
1174(gt)	<i>flaE, K</i>	802(gt)	<i>motA</i>
1199(gt)	<i>flaE, K, motA</i>	813(gt)	<i>motA</i>
1212(a)	<i>flaAIII</i>	aM4(a)	<i>Hr(ah₁)</i>
1215(gt)	<i>flaC, M, motB</i>		

and fewer than in the controls. To examine the correspondence between these subgroups and those of *S. typhimurium*, transductions were carried out between the *S. typhimurium* mutants and the new mutants. The results showed (Table 4a) that *flaAI* corresponds to subgroup of *flaA37*, *flaAII* to that of *flaA23* and *34*, and *flaAIII* to that of *flaA22*. *FlaAII* paralysed mutants were shown to correspond to *motC* mutants of *S. typhimurium*. They were qualitatively different from *motA* and *B* paralysed mutants as will be described later.

All the paralysed mutants and five non-flagellate *flaAII* mutants showed characteristic non-reciprocal complementation (Armstrong & Adler, 1967); that is when used as recipients they always gave shorter and in many cases fewer or no trails even in transduction with wild-type. When used as donors they gave normal trails in normal numbers.

Mutants described as *flaE* did not complement or gave a trail index of only 1 with *flaE60* and *flaJ28* (Table 4b). Since it was possible that the *flaJ* and *flaE* cistrons were the same (Iino, 1969), complementation between *flaE60* and *flaJ28* was re-examined in reciprocal transduction. A trail index of 1 was obtained in both crosses, suggesting that they belonged to the same complementation group designated *flaE*.

Six mutants were not complemented by *Salmonella typhimurium* mutant *ah₁-1*. Because *ah₁* only activates *H₁* in the cis-position (Iino, 1961), *ah₁⁻* and *H₁⁻* mutants should not complement each other, thus making it impossible to distinguish them from each other in complementation tests. In this work these mutants were therefore provisionally classified as *H₁⁻* mutants and designated *aM1*, *aM2*, etc. in accordance with Joys and Stocker (1966) who described the *H₁* with altered phase 1 antigen *i*.

Three groups of non-flagellate mutants did not correspond to any known cistron and were designated *flaN*, *P* and *Q*, respectively. All three *flaN* mutants showed non-reciprocal complementation (Table 4c).

Trails resulting from intracistronic complementation were generally shorter than those from intercistronic complementation.

Non-reciprocal complementation. When used as recipients in complementation tests several single site *flaAII* and *N* mutants gave shorter and, in many cases, fewer trails than those formed when they were donors. Such non-reciprocal complementation has been also found with some *mot* mutants of *Escherichia coli* (Armstrong & Adler, 1967) and some *flaA* and *C* mutants of *Salmonella typhimurium* (Joys & Stocker, 1965). Armstrong & Adler (1967) proposed that defective products of the mutated genes inhibit complementation by being incorporated into a structural component of the cell. If this was so, mutants not producing a defective product, such as those having a large deletion covering most or all of the cistron, would not inhibit trail formation. Transduction was carried out between S3241 (wild-type) as donor and *flaAII* and *flaN* deletion mutants as recipients. All the deletion mutants produced normal trails with an index of 3 except for *fla1293*, whereas single site *flaAII* and *N* mutants examined in parallel produced shorter and fewer trails (Table 5). Trails of *flaN* deletion mutant *fla1293* were shorter than normal, but it was also noted that swarms produced from this mutant spread very slowly as compared with those produced from other recipients, which may mean that the mutant carried some gene causing poor motility even when *flaN* was introduced. These results suggest that the shorter trails with single site mutants were the results of incomplete complementation inhibited by defective products of the mutated genes. The hypothesis of Armstrong and Adler is therefore supported.

*Numbers of flagella of paralysed *flaAII* mutants.* In contrast to other *mot* mutants, paralysed *flaAII* mutants showed variable agglutination with anti-flagella serum and microscopic observation revealed that the numbers of flagella per bacterium differed (Fig. 2). For S3241

Table 4. (Cont.)

(b) <i>flaE</i> and <i>K</i> mutants														(c) <i>flaN</i> , <i>P</i> and <i>Q</i> mutants														
Donor ... Recipient		<i>flaE</i>				<i>flaK</i>				Standards				Donor ... Recipient		<i>flaN</i>		<i>flaP</i>	<i>flaQ</i>							SI241**		
		1152	1162	1171	1186	1159	1190	1194	1195	<i>flaE</i> 60	<i>flaJ</i> 28	<i>flaK</i> 44	1201			1225	1291	1337	1202	1205	1235	1246	1309	1324	1331			
<i>flaE</i>	1152	0	1	0	1	3	3	3	3	0	1	3	3	<i>flaN</i>	1201	0	0	0	2'	1'	2'	3'	1'	2'	2'	2'	2'	
	1162	1	0	0	1	3	3	3	3	1	1	3	3		1225	0	0	0	2'	2'	2'	1'	1'	2'	2'	2'	2'	
	1171	1	1	0	1	3	3	3	3	0	0	3	3		1291	0	0	0	2'	0	2'	2'	1'	1'	2'	2'	1'	
	1186	1	1	1	0	3	3	3	3	0	1	3	3		1337	3	3	3	0	3	3	3	3	3	3	3	3	
<i>flaK</i>	1159	3	3	3	3	0	0	0	1	3	3	1'	3	<i>flaQ</i>	1202	3	3	3	3	0	0	1	0	1	0	0	3	
	1190	3	3	3	3	0	0	0	0	3	3	0	3		1205	3	3	3	3	0	0	0	0	1	0	0	3	
	1194	3	3	3	3	0	0	0	0	3	3	0	3		1235	3	3	3	3	0	0	0	0	1	0	0	3	
	1195	3	3	3	3	0	0	0	0	3	3	0	3		1246	3	3	3	3	0	0	0	0	1	0	0	3	
Control	<i>flaL</i> 1598	3	3	3	3	3	3	3	3	3	3	3	3	Control	1309	3	3	3	3	0	1	1	1	0	1	1	3	
	<i>flaL</i> 1599	3	3	3	3	3	3	3	3	3	3	3	3		1324	3	3	3	3	1	0	1	0	1	0	0	3	
	<i>flaL</i> 1600	3	3	3	3	3	3	3	3	3	3	3	3		1331	3	3	3	3	0	0	0	0	1	1	0	3	
														Control	<i>flaL</i> 1411	3	3	3	3	3	3	3	3	3	3	3	3	3
															<i>flaL</i> 1413	3	3	3	3	3	3	3	3	3	3	3	3	
															<i>flaL</i> 1415	3	3	3	3	3	3	3	3	3	3	3	3	

* Paralysed mutants: their average number of flagella per bacterium vary from 0 to almost the same as wild-type.

** Wild-type.

(wild-type) the number varied from 0 to 8, and for *flaI439* the distribution was similar but with more bacteria with fewer flagella. For *flaI259* and *fla310*, this altered distribution was more marked; more than 70 % of the latter mutants had no flagellum. The average flagellar numbers per bacterium were 2.9 for SJ241, 1.9 for *flaI439*, 0.7 for *flaI259*, and 0.3 for *fla310*. Variation in flagellar length of these mutants was not significantly different from SJ241.

Co-transduction of fla and mot cistrons with H_I. Transduction was carried out from SJ241 (*H_I-a*) to the mutants with unexpressed *H_I-gt* or from TR6 (*H_I-gt*) to those with unexpressed *H_I-a*. Motile clones with the donor phase 1 antigen were recombinants which had received both the donor *H_I* allele and that of the mutant cistron of the recipient, while those with the recipient antigen were recombinants for the latter only. The co-transduction frequency was the ratio of donor-type clones to the total number of recombinants (Table 6). All the mutants examined except *flaMI467* were co-transducible with *H_I*. The frequencies obtained show that the relevant cistrons are separate from *H_I* and in the following order: (*flaAI*, *flaL*) *flaAII* *flaAIII* (*flaB*, *flaD*, *flaN*, *flaP*, *flaQ*) (*flaC*, *flaE*, *flaK*, *motA*, *motB*) *flaM*. The frequency of co-transduction between mutants of bracketed cistrons did not differ significantly from each other.

Mapping of fla and mot cistrons closely linked to H_I with deletions and in three-factor reciprocal crosses. The *Salmonella typhimurium* mutants used in the complementation tests were also included in the mapping. First, it was determined which single site mutants were covered by each deletion. On this basis the mutants fell into two groups, one comprized

Table 5. *Trail formation in transduction between deletion and single site flaAII and N mutants as recipients and wild-type as donor*

Wild-type SJ241 was used. A known volume of the transduction mixture was brushed in line on semi-solid medium. After 20 h incubation, the numbers of trails formed were counted. Degree of trail formation was expressed as in Table 4.

	Mutant no.	Mutated region	Trail formation	
			No. of trails	Trail index
Deletion mutants	306	<i>flaAII</i>	552	3
	1383	<i>flaAI</i> , <i>AII</i>	372	3
	286	<i>flaAII</i>	503	3
	227	<i>flaAI</i> , <i>AII</i>	325	3
	1330	<i>flaAII</i>	378	3
	1336	<i>flaAII</i>	332	3
	1346	<i>flaB</i> , <i>D</i> , <i>N</i> , <i>P</i> , <i>Q</i>	480	3
	375	<i>flaB</i> , <i>D</i> , <i>N</i> , <i>P</i> , <i>Q</i>	420	3
	1293	<i>flaN</i> , <i>P</i>	356*	3
	1442	<i>flaAII</i>	0	0
Single site mutants	1247	<i>flaAII</i>	0	0
	1259	<i>flaAII</i>	0	0
	1296	<i>flaAII</i>	4*	1
	1332	<i>flaAII</i>	8*	1
	1305	<i>flaAII</i>	35*	2
	1201	<i>flaN</i>	5*	1
	1225	<i>flaN</i>	110*	2
	1291	<i>flaN</i>	10*	1
	1411	<i>flaL</i>	449	3
Control	1413	<i>flaL</i>	422	3
	1415	<i>flaL</i>	435	3
			Av. 435	

* Trails shorter than control.

Fig. 2. The numbers of flagella of paralysed *flaAII* mutants of *Salmonella abortus-equi*.

Table 6. Co-transduction frequency of *fla* and *mot* mutants with *Hr*

Transductions were carried out from TR6 (*Hr-gt*) to non-motile mutants of SJ241 (*Hr-a*), and from SJ241 to non-motile mutants of TR6. Motile recombinants appearing as swarms in semisolid medium were isolated and their flagellar antigens typed by slide-agglutination. When the co-transduction frequency was less than 1 %, serial tenfold dilutions of the transduction mixture were brushed on semisolid medium without serum and with antiserum specific for the recipient-type flagellar antigen. Swarms appearing on the latter medium were designated donor-type recombinants. Co-transduction frequency was the percentage of donor-type transductants amongst the recombinants.

Donor		Recipient			Motile recombinants			Co-transduction frequency (%)	99.7 % confidence interval ($\pm$ %)
Strain no.	<i>Hr</i> allele	Mutant no.	Mutated cistron	<i>Hr</i> allele	Donor-type	Recipient-type	Total		
TR6	<i>Hr-gt</i>	<i>aMr</i>	<i>Hr</i>	<i>a</i>	660	0	660	100.0	0.3
		1413	<i>flaL</i>	<i>a</i>	336	3	339	99.1	1.5
		1310	<i>flaAI</i>	<i>a</i>	718	2	720	99.7	0.6
		310	<i>flaAII</i>	<i>a</i>	393	137	530	74.1	5.7
		332	<i>flaAIII</i>	<i>a</i>	171	223	394	43.4	7.4
		1291	<i>flaN</i>	<i>a</i>	81	549	630	12.8	4.0
		1337	<i>flaP</i>	<i>a</i>	98	538	636	15.4	4.2
		1235	<i>flaQ</i>	<i>a</i>	61	462	523	11.6	4.2
		1256	<i>flaB</i>	<i>a</i>	41	404	445	9.2	4.1
		1301	<i>flaD</i>	<i>a</i>	52	461	513	10.1	3.9
		1152	<i>flaE</i>	<i>gt</i>	31	—	88×10^3	0.035	0.018
		1186	<i>flaE</i>	<i>gt</i>	29	—	93×10^3	0.031	0.017
		1158	<i>flaK</i>	<i>gt</i>	18	—	70×10^3	0.025	0.018
SJ241	<i>Hr-a</i>	1159	<i>flaK</i>	<i>gt</i>	24	—	71×10^3	0.033	0.020
		811	<i>motA</i>	<i>gt</i>	20	—	75×10^3	0.026	0.018
		815	<i>motA</i>	<i>gt</i>	23	—	57×10^3	0.040	0.025
		816	<i>motB</i>	<i>gt</i>	21	—	70×10^3	0.030	0.019
		810	<i>motB</i>	<i>gt</i>	11	—	51×10^3	0.021	0.019
		1143	<i>flaC</i>	<i>gt</i>	9	—	68×10^3	0.013	0.013
		1146	<i>flaC</i>	<i>gt</i>	17	—	66×10^3	0.025	0.018
		1467	<i>flaM</i>	<i>gt</i>	0	—	72×10^3	0.00	—

flaAI, *AII*, *AIII*, *B*, *D*, *L*, *N*, *P*, *Q*, and *H1* (*ah₁*) mutants, and the other *flaC*, *E*, *K*, *M*, *motA* and *B*. In further deletion mapping (Fig. 3) the orders of the cistrons in each group were determined to be *-flaD-flaB-[flaQ, (flaN, flaP)]-[flaAIII, (flaAII, flaAI)]-(H1, flalL)-*, and *-[(flaE, flak), motA]-motB-flaC-flaM-*. The relative orders of bracketed cistrons were not determined.

Next three-factor reciprocal crosses between pairs of non-motile mutants using *H1* as the unselected marker were carried out to determine the orders of the sites within and between deletions. Pairs of non-motile mutants with *H1* alleles different from each other were used and the resulting motile recombinants examined for their flagellar antigens by slide-agglutination. Consider the reciprocal crosses between *fla-a H1-A* and *fla-b H1-B*. If the sequence is *fla-a-H1-fla-b* (Table 7*a*), both the A- and B-type transductants require two crossovers regardless of the direction of the cross. Since the ratio of the B- to A-type transductants in either cross depends on the crossover frequencies in regions 2 and 3 shown in Table 7 (*a*), the B/A ratios should be the same in both crosses although the absolute numbers may be different depending upon the length of regions 1 and 4. In contrast, if the sequence is *fla-a-fla-b-H1*, the B/A ratios in the reciprocal crosses should not be the same because, with *fla-a H1-A* as donor (cross 3 in Table 7*b*), motile transductants of either type require two crossovers, whereas in the reverse cross (cross 4 in Table 7*b*) B-type transductants require four crossovers in the regions 1 to 4, and A-type transductants require only two in regions 1 and 2, i.e. the number of B-type transductants will be much less than those of A-type in this cross. Thus, the B/A ratios in reciprocal crosses should give the relative order of the mutation sites with respect to *H1*.

The following sequences were compatible with the results of three-factor cross tests (Table 8): *-flaAIII-flaAII-flaAI-H1-flaL-*, *-flaQ-flaP-flaN-H1-*, and *-flaD-flaB-H1-*. Putting together these results and those of the deletion mapping, the following order is suggested: *-flaD-flaB-flaQ-flaP-flaN-flaAIII-flaAII-flaAI-H1 (ah₁)-flaL-*. The three-factor cross was not used to determine the order of *flaC*, *E*, and *K*, and *motA* and *B* mutation sites because of their low frequencies of co-transduction with *H1*. A genetic map representing all these results appears in Fig. 3. It should be noted that all the paralysed mutants of *flaAII* and *Salmonella typhimurium motC* mutants were located at the left end of *flaAII*. It would be also noted

Table 7. Principle of the three-factor cross test between pairs of non-motile mutants using *H1* as the unselected marker

Sequence	Reciprocal transduction	Motile recombinants		
		Cross-over region	Anti-gen type	Ratio of the B- to A-type recombinants
(a) <i>fla-a-H1-fla-b</i>	Cross 1			
	Donor <i>-fla-a-H1-A-+-</i>	{ 2, 4	A	Depending upon crossing over in regions 2 and 3, the ratio of B/A being theoretically equal in both crosses
	Recipient ¹ <i>-+²H1-B³-fla-b⁴</i>		B	
	Cross 2			
	Donor <i>-+-H1-B-fla-b-</i>	{ 1, 2	A	
	Recipient ¹ <i>-fla-a²-H1-A³+⁴</i>		B	
(b) <i>fla-a-fla-b-H1</i>	Cross 3			
	Donor <i>-fla-a-+-H1-A-</i>	{ 2, 4	A	The B-type in cross 4 requires four crossovers, so that the ratio of B/A in cross 4 will be much less than that in cross 3
	Recipient ¹ <i>-+²-fla-b³-H1-B⁴</i>		B	
	Cross 4			
	Donor <i>-+-fla-b-H1-B-</i>	{ 1, 2	A	
	Recipient ¹ <i>-fla-a²+³-H1-A⁴</i>		B	

that deletions *fla375* and *fla1346* both covering *flaD* to *N* overlap with deletions *fla1238* and *fla1319* covering *flaAIII* to *AI* and *flaAIII* to *L*, respectively (Fig. 3), suggesting that an additional *fla* cistron exists between *flaAIII* and *N*.

DISCUSSION

The three *Salmonella abortusequi* cistrons *flaN*, *flaP* and *flaQ* have not been detected in *S. typhimurium*. This may not reflect any basic difference between the species, since motile strains of *S. typhimurium* could complement *S. abortusequi flaN*, *P* and *Q* mutants, suggesting that these cistrons exist in both species.

It was shown that the *fla* and *mot* cistrons closely linked to *Hr* form at least two clusters. The sequence of cistrons in the cluster comprising *flaC*, *E*, *K* and *M*, and *motA* and *B* was

Table 8. *Determination of the sequence of fla sites with respect to Hr in three-factor reciprocal crosses*

Reciprocal transduction was carried out between pairs of *fla* mutants carrying different *Hr*-alleles. *fla*⁺ recombinant swarms on semisolid medium were isolated and their antigens determined by slide-agglutination. The sequence of mutation sites with respect to *Hr* was in accordance with the principles of Table 7.

Reciprocal transduction			<i>fla</i> ⁺ recombinants			Order inferred
Pair of mutants*	Donor	Recipient	Antigen type		Total	
			<i>a</i>	<i>gt</i> or <i>fg</i>		
{ 306 (<i>AII</i> , <i>a</i>) and 204 (<i>L</i> , <i>fg</i>)	306	204	130	82	212	306— <i>Hr</i> —204 (<i>AII</i>) (<i>L</i>)
	204	306	189	95	284	
{ 310 (<i>AII</i> , <i>a</i>) and 227 (<i>AI</i> , <i>AII</i> , <i>fg</i>)	310	227	162	31	193	310—227— <i>Hr</i> (<i>AII</i>) (<i>AI</i> , <i>AII</i>)
	227	310	241	0	241	
{ 227 (<i>AI</i> , <i>AII</i> , <i>fg</i>) and 1328 (<i>AIII</i> , <i>a</i>)	227	1328	116	0	116	1328—227— <i>Hr</i> (<i>AIII</i>) (<i>AI</i> , <i>AII</i>)
	1328	227	157	71	228	
{ 220 (<i>AIII</i> , <i>fg</i>) and 306 (<i>AII</i> , <i>a</i>)	220	306	0	120	120	220—306— <i>Hr</i> (<i>AIII</i>)(<i>AII</i>)
	306	220	21	64	85	
{ 1251 (<i>AIII</i> , <i>a</i>) and 220 (<i>AIII</i> , <i>fg</i>)	1251	220	45	123	168	1251—220— <i>Hr</i> (<i>AIII</i>) (<i>AIII</i>)
	220	1251	202	1	203	
{ 220 (<i>AIII</i> , <i>fg</i>) and 1234 (<i>AIII</i> , <i>a</i>)	220	1234	1	179	180	1234—220— <i>Hr</i> (<i>AIII</i>) (<i>AIII</i>)
	1234	220	31	192	223	
{ 1148 (<i>N</i> , <i>gt</i>) and 1337 (<i>P</i> , <i>a</i>)	1148	1337	171	1	172	1337—1148— <i>Hr</i> (<i>P</i>) (<i>N</i>)
	1337	1148	19	160	179	
{ 1147 (<i>Q</i> , <i>gt</i>) and 1293 (<i>N</i> , <i>P</i> , <i>a</i>)	1147	1293	195	16	211	1147—1293— <i>Hr</i> (<i>Q</i>) (<i>N</i> , <i>P</i>)
	1293	1147	158	0	158	
{ 1337 (<i>P</i> , <i>a</i>) and 1147 (<i>Q</i> , <i>gt</i>)	1337	1147	0	221	221	1147—1337— <i>Hr</i> (<i>Q</i>) (<i>P</i>)
	1147	1337	107	27	134	
{ 1245 (<i>B</i> , <i>a</i>) and 211 (<i>D</i> , <i>fg</i>)	1245	211	0	191	191	211—1245— <i>Hr</i> (<i>D</i>) (<i>B</i>)
	211	1245	140	27	167	

* Mutated cistron and *Hr* allele of the mutant are shown bracketed.

not completely determined in the present study. As no co-transduction of *flaM* and *Hr* was detected *flaM* may be the most distal cistron from *Hr*.

Hr and *Hr*-linked *fla* and *mot* are known to be located between *his* and *tre* on the chromosome (summarized in Sanderson, 1970). Enomoto (1966*b*) reported that *motC* lies on the *his* side of *Hr* with *motA* and *B* on the opposite side. The same author isolated a deletion covering the whole of *motB* and a part of *motA* but no *fla* gene (Enomoto, 1966*a*). From results of three-factor cross tests D. B. Fankhauser (personal communication) deduced that *supW* is located between *his* and *fla*. Stocker, McDonough & Ambler (1961) mapped *nml*, a gene determining presence or absence of ϵ -*N*-methyllysine in flagellin, between *Hr* and *flaA*. Combining these results and ours with *Salmonella abortusequi*, the following order of cistrons on *Salmonella* genomes is inferred: *-his-supW-flaD-flaB-flaQ-flaP-flaN-flaAIII-flaAII-flaAI-nml-Hr(ah₁)-flaL-(flaE, flaK)-motA-motB-flaC-flaM-tre-*. The relative positions of *flaB* and *flaD* in *S. typhimurium* are not yet certain, as Joys & Stocker (1969) reported the order *-flaB-flaD-flaA-Hr-*, but D. B. Fankhauser (personal communication) obtained results supporting the order *-flaD-flaB-flaA-*.

Several single site *flaAII* and *N* mutants showed non-reciprocal complementation. Armstrong & Adler (1967) offered two possible explanations of the same phenomenon between *mot* mutants of *Escherichia coli*. The first was that it could be a gene dosage effect; the partial heterozygote containing only one donor chromosomal fragment but more than two recipient chromosomes. The second was that complementation is inhibited if defective subunits are incorporated into a structural component of the cell. Our results support the latter explanation, because deletion mutants of the relevant cistrons, presumably producing no defective products, showed normal complementation. If a critical level of the defective products is necessary to inhibit normal flagellum formation, the first explanation could be compatible with the production of normal flagella when these single site mutants are used as donors.

The bacterial flagellum consists of at least three parts, a filament composed of flagellin monomers, a basal body, and a hook between them (Kerridge, Horne & Glauert, 1962; Glauert, Kerridge & Horne, 1963; Hoeniger, 1965; Lowy, 1965; Abram, Vatter & Koffler, 1966; Cohen-Bazire & London, 1967). The basal structure including the hook is now thought to include the site of flagellin synthesis and the starting-point of flagellin polymerization into a flagellum and to play some role in flagellar movement. Iino & Lederberg (1964) proposed two alternative hypotheses concerning the function of the *fla* genes. One was that *fla* controls the production of flagellosomes, hypothetical ribosome systems specializing in flagellin synthesis, and the other that *fla* produces an internal inducer of flagellin synthesis. Later, the first hypothesis was modified by the suggestion that *fla* controls the production of a component of the whole flagella-forming apparatus equivalent to the basal structure (Iino, 1969). The occurrence of both paralysis and decrease in flagellar number following mutation in the *flaAII* region strongly suggests that the product of the cistron is a component of the basal structure which is concerned with both flagellar formation and motility.

The complementation pattern between *flaA* suggested the existence of functional subgroups. If the cistrons corresponding to the subgroups are parts of an operon and their products form a complex on the polysome immediately after translation, it could be that the products of the genes in the cis-position are incorporated into the complex in preference to those in the trans-position. For genes determining subunits in a complex, clustering could be of advantage in the effective formation of the complex, since diffusion of the gene products after translation would be avoided. The function of polysomes as sites of both protein synthesis and subunit aggregation has been suggested for β -galactosidase synthesis in *Escherichia coli* (Zipser & Perrin, 1963).

We are grateful to Dr B. A. D. Stocker of Stanford University for supplying several *fla* mutants of *Salmonella typhimurium*, and to Professor T. Taira of Waseda University for his encouragement and helpful discussion. Contribution from the National Institute of Genetics, Japan, no. 863.

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