NONSENSE MOTILITY MUTANTS IN *SALMONELLA TYPHIMURIUM*[1]

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Manuscript received August 2, 1972
Revised copy received October 17, 1972
Transmitted by DAVID SCHLESSINGER

ABSTRACT

Of 313 motility-deficient mutants isolated from an LT2 *his*(amber) strain fixed in phase 1 by gene *vh2*$^-$, 25 regained motility when amber or ochre suppressors were introduced, in F′ factors or by transduction. The *fla* mutants (23 amber, 1 ochre) fell in complementation groups A, B, C, F, K, a new group, M, and at least one further new group; the hypothesis of a *fla* gene which specifies only an RNA structural component of a flagellum-synthesizing basal apparatus is disproven for the corresponding genes. Hfr and transductional crosses confirmed gene assignments from complementation and indicated that *flaM* and another new *fla* locus map near *H1*. A small minority of motile bacteria were detectable in many of the amber *fla* mutants. In groups A and F some pairs of amber *fla* mutants complemented each other, and perhaps each of these groups corresponds to more than one structural gene. The suppressed derivatives of a mutant with an amber mutation in *H1* made flagella morphologically and serologically indistinguishable from wild-type flagella. A slow-spreading but flagellate mutant showed mainly non-translational motility in broth, and in a viscous medium the bacteria reversed very frequently; its amber mutation, probably near *H1*, is inferred to cause a defect in chemotaxis, so that the bacteria give the avoidance reaction continuously.

IN *Salmonella typhimurium* three classes of genes affecting motility have been investigated: (1) The *H* genes, *H1* and *H2*, the structural genes for the phase-1 and phase-2 flagellins; (2) the *fla* genes, needed for production of flagella; (3) the *mot* genes, needed for their motor function (for review see IINO 1969). A further class, the chemotaxis-deficient or *che* mutants, investigated in *Escherichia coli*, spread only very slowly in semisolid medium, though the bacteria appear normally motile by microscopy (ARMSTRONG, ADLER and DAHL 1967).

Complementation of motility mutants is tested by the production of trails, i.e., by abortive transduction of motility, and many *fla* complementation groups have been described in *S. typhimurium* (JOYS and STOCKER 1965; IINO and ENOMOTO 1966). The loci of mutants of groups A, B, C, D, E, J, K and L lie between *his*

[1] This paper is based on the Ph.D. thesis (Stanford University, 1969) of P. S. VARY. The investigation was supported by Public Health Service research grant AI 07168 and training grant AI-82, both from the National Institute of Allergy and Infectious Diseases.

[2] P. S. VARY held a predoctoral fellowship from the Public Health Service, Number GM-19,998.

and *trp*, and *H1* is co-transduced with all or nearly all of them. The group F locus is between *gal* and *trp*. Each *fla* complementation group presumably corresponds to an *fla* gene, but the immediate functions of the *fla* genes are not known. In *S. typhimurium* flagellin is incorporated into flagella as soon as it is synthesized, without passing through a cytoplasmic pool of detectable size (KERRIDGE 1963). It has been surmised that in *Salmonella* flagellin is synthesized by specialized ribosomes at the base of the flagellum, and that some *fla* genes may code not for proteins but for RNA structural components of the hypothetical synthesizing basal organelle or "flagellosome" (IINO and LEDERBERG 1964). As nonsense mutations are expressed only when an RNA molecule is used as "message" for synthesis of a polypeptide chain, the assignment of a nonsense *fla* mutant to a complementation group would show that the corresponding *fla* gene coded for a protein, not only for an RNA species. In this paper we describe the isolation and complementation properties of a number of nonsense *fla* mutants. In wild-type (diphasic) *S. typhimurium* strains mutation in one of the two flagellin structural genes will not cause permanent loss of motility, because such mutants can regain motility by change of phase, a frequent event. We therefore introduced gene *vh2*$^-$, which prevents change of phase (IINO 1961b), so that mutation at any *fla* or *mot* locus or at the expressed *H* locus would cause loss of motility; mutation at a *che* locus would cause inability to spread in semisolid medium. We shall use "motility mutants" to denote all such classes. Suppressibility of motility mutations was tested by introducing amber or ochre suppressor genes, in F′ factors or by transduction, with selection for suppression of an amber *his* mutation. The nonsense motility mutants we isolated included, in addition to *fla* mutants, an amber *H1* mutant and an amber mutant showing anomalous motility, probably resulting from a defect in the mechanism of chemotaxis.

MATERIALS AND METHODS

Strains and phages: The main strains used are listed in Table 1. Strains of known complementation group, tested for ability to complement all the nonsense motility mutants, comprised those indicated in Tables 4 and 5 and also *flaD58*, *flaE60*, *flaG100*, *flaJ28*, *motA257*, *motB215*, and *motC244* (JOYS and STOCKER 1965; IINO and ENOMOTO 1966; ENOMOTO 1966). Phage P22.L4 (*int*$^-$) (SMITH and LEVINE 1967) was used for transduction and phage χ (MEYNELL 1961) for selection of non-motile mutants. Amber phage mutants used were P22.*n2c* (KOLSTAD and PRELL 1968) and Felix O.*am1* (HEDGES 1971).

Isolation of motility mutants: Three mutagens were used: (1) Ethyl methane sulfonate (LOVELESS and HOWARTH 1959); (2) 2-aminopurine, by the method of C. YANOFSKY (pers. comm.). Tubes containing 0.5 mg 2-aminopurine in 0.5 ml of broth were inoculated with ca. 100 cells of a log-phase culture and shaken at 36°C for 24 hours, then turbid tubes were diluted tenfold in broth and shaken for a further 4 hrs; (3) N-methyl-N′-nitro-N-nitroso-guanidine, at 0.1 mgm/ml (ADELBERG, MANDEL and CHEN 1965). Two methods were used for isolation of motility mutants from mutagen-treated cultures: (i) Selection by χ phage (JOYS and STOCKER 1965); (ii) Picking non-spreading colonies from pour plates of semisolid medium. Only apparently non-leaky, reasonably stable motility mutants, unaltered in nutritional character, were retained.

Transduction and complementation: Phage P22.L4 was added, to multiplicity ca. 10, to an overnight broth culture of the recipient strain. After 15 min, samples were plated for selection of nutritional transductants, and standard loopful amounts (ca. 0.003 ml) were spread on semisolid medium; swarms and trails were counted after overnight incubation at 36°C. Slide agglutination was used to test the flagellar antigen(s) of *fla*+, etc., transductant clones.

TABLE 1

Main strains used*

Strain number	Description	Source and/or reference
	(a) Starting strains	
LS322	*E. coli* K12 *pro trpA* (amber) *his* (amber) *ilv trpR carrying* F'14 *ilv*+ *su7* (amber suppressor)	*su7* mutant of LS302 (SOLL and BERG 1969)
SA904	LT2 *proA26* (P22) Hfr, injecting in sequence ← *trp* — *his* —	K. E. SANDERSON (pers. comm.)
SB458 (≡TA131)	LT2 *hisC527 supD501* (amber suppressor)	BERKOWICZ *et al.* 1968
SB789	LT2 *metA trpB H1-b H2-e,n,x* "cured of Fels 2" *str fla rfb* carrying F'13 *lac*-U281 (amber)	BERKOWICZ *et al.* 1968
SL789	LT2 *hisD27 trpA8*	Received from P. E. HARTMAN
SL1027	LT2 *metA22 trpB2 H1-b H2-e,n,x* "cured of Fels 2" *fla strA xyl metE*	KUO and STOCKER 1970
SL3547 (=re-isolate of SA22)	LT2 *proA26* (P22) HfrB2 *tre*+ *clb*+ *galE446*	SANDERSON (1970) and pers. comm.
SL3625	LT2 *hisC527* (amber) *xyl-416 fla-401* (amber)	xylose-negative mutant obtained from LT2 *hisC527* by ethyl methane sulfonate treatment
SL4040	LT2 *metA trpB H1-b H2-e,n,x* "cured of Fels 2" *fla str gal* carrying F'1 *gal*+ *sup-812* (ochre suppressor)	F' factor derived from SL4528 (MACPHEE and STOCKER 1969)
SL4528	M7471 (colE1) *leu malB cysI hisC527* (amber) *gal* carrying F'1 *gal*+ *sup-812* (ochre suppressor)	MACPHEE and STOCKER 1969
	(b) Derived strains	
SL4001	as SL3625 but *sup-811* (amber suppressor)	Spontaneous suppressor mutant of SL3625 obtained by selection in semisolid medium
SL4012†	LT2 *trpA8 hisC527* (amber)	Obtained from SL789 by transducing in *hisD*+ *hisC527* (amber) from LT2 *hisC527*
SL4013	LT2 *trpA8 hisC527* (amber) *H2-e,n,x vh2*− ("fixed" phase-2 clone, expressing *H2-e,n,x*)	Obtained from SL4012 by transducing in *H2-e,n,x vh2*− from UP186 (U. PEARCE, University of London Ph.D. thesis, 1965)
SL4018†	LT2 *trpA8 hisC527* (amber) *H2-e,n,x vh2*− ("fixed" phase-1 clone, expressing *H1-i*)	Obtained from SL4013 by rare change of phase
SL4021	as SL4018 but *strA*	Obtained from SL4018 by transducing in *strA* from SL1027
SL4049†	as SL4021 but *gal-489* (amber)	Obtained from SL4021 by ethyl methane sulfonate exposure

* All strains except LS322 are *S. typhimurium*. Singly marked strains are referred to by mutation symbol only. Strains used as representatives of known *fla* and *mot* complementation groups are listed in METHODS section of text and in Tables 4 and 5. Strains with prefix SA are from culture collection of K. E. SANDERSON, with prefix SB from collection of P. E. HARTMAN, with prefix SL or SD from collection of B. A. D. STOCKER.

† Motility mutants were isolated from cultures of strains SL4012, SL4018 and SL4049 (see Figure 1).

To test for expression of the *H1-i* allele of mutant #521 in phase-1 abortive transductants a culture of this strain was treated with phage grown on an *H1-b H2-e,n,x vh2*+ *fla*+ donor and inoculated on semisolid agar containing anti-*e,n,x* serum, alone or with anti-*i* serum; and cells of an *H1-b H2-e,n,x flaA*− recipient, SL696, in (latent) phase 1 were treated with phage grown on mutant #521 and similarly tested (see PEARCE and STOCKER 1965, 1967 for this technique). To test for complementation in respect of ability to make phase-1 flagella a phase-1 (and therefore non-motile) culture of an *ah1*− *H1-i H2-1,2* strain, SW1061, was treated with phage grown on mutant #521 and spread on semisolid medium containing anti-*1,2* serum (IINO 1961a). Appropriate control strains gave the expected result in all these tests.

Introduction of suppressors: The ochre suppressor *sup-812*, in an F'1-*gal* factor (MACPHEE and STOCKER 1969), and the amber suppressor *su7*, in an F'14 *ilv*+ factor (SOLL and BERG 1969), were transferred to motility mutants by conjugational crosses from *S. typhimurium* or *E. coli* donors (Table 1), selection being made for His+, i.e., for suppression of *hisC527* (amber) of the recipient. Amber suppressors *supD501* (FANKHAUSER and HARTMAN 1971) and *sup-811* (see RESULTS) were introduced by transduction from LT2 *hisC527 sup* donors (Table 1), with selection for His+. (In such *hisC527* → *hisC527* crosses only a few His+ colonies arose by "selfing" (DEMEREC 1963)). Suppressor-bearing derivatives of motility mutants after purification were tested for motility, by microscopy and by incubation in semisolid medium; when necessary their suppressor status was checked by crossing to an F'*lac* (amber) donor (Table 1) and by testing sensitivity to amber phage mutants.

Microscopy: Young broth cultures were examined by low-power microscopy with dark-field illumination; a counting chamber was used for semi-quantitative estimation of the very small number of motile cells present in some cultures. Flagellar staining was by the method of LEIFSON (1951), or by that of RYU (1937), which does not require acid-cleaned slides.

RESULTS

Recognition of a suppressible fla mutation and of an amber suppressor: Strain SL3625, a mutagen-induced *xyl* mutant of LT2 *hisC527* (amber) (Table 1), spread very slowly in semisolid medium and only 5%–15% of cells in broth cultures were motile. Repeated passage through semisolid medium yielded a rapid-spreading variant, SL4001, which no longer required histidine. A possible explanation was that the slow-spreading SL3625 carried a leaky suppressible *fla* mutation and that its rapid-spreading variant, SL4001, arose by a suppressor mutation, active on the amber *his* and the *fla* mutations. Transduction of the *his* region of SL4001 to the deletion mutant *hisFAHBCD152* gave numerous colonies on histidinol-supplemented medium, none (or almost none) of which grew on medium without histidinol—as expected if they had acquired *hisD*+ *hisC527* from a *hisC527* strain carrying an external suppressor. The mutation, *sup-811*, was an amber suppressor by the following criteria: (1) it suppressed the F' *lac* (amber) mutation; (2) it suppressed the amber phage mutations, P22.*n2c* and Felix 0.*am1*; (3) when transduced to *hisC* nonsense mutants it suppressed all of several amber mutants, but none of four ochre and one UGA mutant.

We tested for P22 co-transduction of the new suppressor, *sup-811*, and *trp*, *gal* or *his*, with negative results (Table 2) (cf. EGGERTSSON and ADELBERG 1965). FANKHAUSER and HARTMAN (1971) report that phage P22 co-tranduces *flaA*, *flaB* and *flaD* with an amber suppressor, *supD501*, at frequencies of 17%–40%. We observed co-transduction of *supD501*, but not of *sup-811*, with *flaA* (Table 2). The amber *gal-489* (present in many of the motility mutants) was well sup-

TABLE 2

Co-transduction tests, using phage P22.L4*

Donor†	Recipient†	Donor allele		Rate of co-transduction§
		Selected	Unselected‡	
SL4001, *sup-811 hisC527* (amber)	SL4012, *trpA*⁻ *hisC527* (amber)	*trp*+	*sup-811*	0/330
		sup-811	*trp*+	0/392
SL4096, *sup-811 hisC527* (amber) *galE*⁻	SL4044, *hisC527* (amber)	*sup-811*	*galE*⁻	0/299
SL4001, *sup-811 hisC527* (amber)	LT2 *hisC129* (deletion)	HIS+, i.e. *hisC527* (amber) and *sup-811*	—	0/ca. 500
SJ373, *flaA41* (deletion)	SL4062, *trpA*⁻ *hisC*⁻ *flaM*⁻	*trp*+	*flaM*+	0/25
		his+	*flaM*+	0/25
SB458, *supD501*	SJ373, *flaA41* (deletion)	*fla*+	*supD501*	3/17
SL4096, *sup-811*	SJ373, *flaA41* (deletion)	*fla*+	*sup-811*	0/43

* For tests of co-transduction of *H1* with *fla*+, etc., see last column of Table 3.

† Only relevant items of donor and recipient genotype listed; for complete genotypes see Tables 1 and 3.

‡ Presence of unselected donor allele *sup-811* or *supD501* was inferred from histidine-independence in case of *hisC527* (amber) recipients, and from sensitivity to amber Felix 0 phage, for other recipients.

§ Number of transductant clones with unselected donor allele/total number of transductants tested; in the case of the cross to the deletion mutant *hisC129* no histidine-independent colonies (i.e., with donor alleles *hisC* (amber) and *sup-811*) were obtained from a sample which yielded ca. 500 colonies in a control cross, with an *his*+ donor.

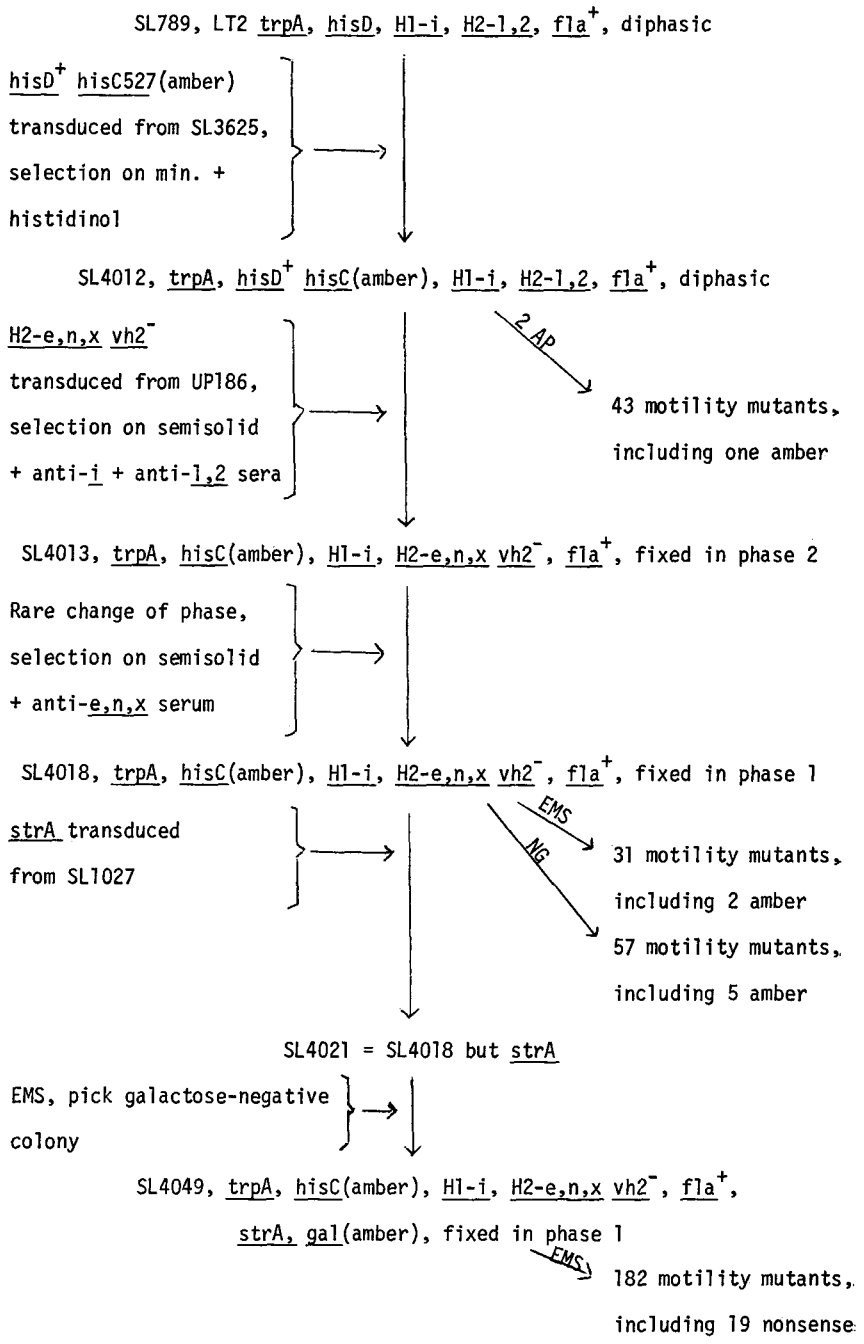

FIGURE 1.—Derivation of motile parent strains and motility mutants.

pressed by *supD501* but only slightly by *sup-811*. Thus the *S. typhimurium* chromosomal amber suppressors *sup-811* and *supD501*, both used to test suppressibility of motility mutations, are non-identical.

Isolation of motility mutants and screening for suppressibility: A series of derivatives were made by transduction, mutation, etc. (Figure 1) from a fully motile LT2 line with markers on each side of the *H1-fla* region, i.e., *his* and *trp*. All the derivatives were sensitive to phage P22, but from SL4013 onwards they were partly sensitive to the rough-specific phage P221.*c2* (WILKINSON, GEMSKI and STOCKER 1972), perhaps because of a partial defect in lipoplysaccharide structure, and also sensitive to phage ES18, perhaps through loss of prophage Fels 2 (KUO and STOCKER 1970).

The mutagen-treated cultures of the derivatives used gave 4%–7% non-spreading colonies in semisolid medium, and from such colonies or by selection with χ phage we obtained 312 reasonably stable, apparently non-leaky non-motile mutants and one non-spreading but motile mutant. They were assigned numbers, *fla*, *mot*, etc., in a single series starting from 401. Negative slide-agglutination tests with anti-H sera showed that the nonmotile mutants lacked flagella, except for 48 with antigen *i*, inferred to be *mot* mutants. The F′1 factor carrying ochre-suppressor *sup-812* (MACPHEE and STOCKER 1969) was introduced into all 313 mutants, and the F′14 factor carrying amber-suppressor *su7* (SOLL and BERG 1969) into most of them; 19 mutants appeared suppressible. Transductional screening with amber-suppressor *sup-811* (see above) showed a further six mutants to be suppressible.

Properties of suppressible motility mutants: The properties of the suppressible mutants are recorded in Table 3. Of the 25 mutants in the *trp his* line only the non-spreading, motile mutant, #411, was agglutinated by anti-*i* serum, and flagellate cells were seen in stained preparations only in this mutant. Though the other 24 mutants had been judged non-leaky during the screening procedure, microscopy of log-phase broth cultures of five mutants showed from ca. 0.01% to ca. 1% of motile cells (Table 3). In pour plates of very soft semisolid medium satellite microcolonies (QUADLING and STOCKER 1957) developed round the colonies of these five mutants, and of six others (Table 3); anti-*i* (but not anti-*e,n,x*) serum inhibited their development. To test reversion, streak inocula of the nonsense motility mutants on semisolid nutrient medium were spotted with mutagens. A few spontaneous motile revertants were observed after overnight incubation at 36°C. No mutant responded to the frame-shift mutagen, compound ICR-191, and all produced swarms in response to base-change mutagens, 2-aminopurine, ethyl methane sulfonate and nitrosoguanidine; most such revertants tested were found to result from suppressor mutations.

All but one of the mutants responded to at least one of the amber suppressors; the exception, *fla-682*, is inferred to be ochre. The motility of suppressor-bearing derivatives varied between mutants and according to suppressor; in some combinations a weak suppression (variable between experiments) was shown only by the presence of a small minority of motile cells, absent in the unsuppressed parent. In other combinations 15% to 85% of the cells were motile (cf. > 95% in

TABLE 3

Origin and properties of nonsense motility mutants

Mutant			Origin		Leakiness		Motility in presence of *suppressor*†				Linkage‡		*H1* co-trans-transduction§
Allele number	Strain number	Complementation group	Parent	How obtained*	Satellites	Microscopy	*sup-812* (ochre)	*sup-811* (amber)	*supD501* (amber)	*su7* (amber)	SL3547	SA904	
fla-401	SL3625	fla A	hisC527	EMS	++++	ca. 10%	+	+	+	n.t.	5/7		0.02
che-411	SL4041	unique	SL4012	2AP,S-S			+	+	+	n.t.	4/8		0/50
fla-466	SL4043	fla F	SL4018	EMS,χ	—	—	+	+	+	n.t.	0/8		0/12
fla-511	SL4118	fla K	SL4018	NG,χ	—	—	(—)	+	+	(—)		12/20	$<10^{-3}$
fla-513	SL4044	fla K	SL4018	EMS,χ	—	—	+	+	+	n.t.	5/9		0/20,$>10^{-3}$
H1-521	SL4045	H1	SL4018	NG,χ	—	—	+	+	+	n.t.	9/9		29/31
fla-526	SL4046	fla A	SL4018	NG,χ	—	—	+	+	+	n.t.	3/4		1/13
fla-530	SL4047	fla F	SL4018	NG,χ	—	—	+	+	+	+	0/10		0/12
fla-546	SL4119	fla C	SL4049	EMS,S-S	—	—	(—)	+	+/±‖	(—)		14/20	$<10^{-3}$
fla-560	SL4052	fla F	SL4049	EMS,χ	—	—	+	+	+	+	0/5		0/12
fla-561	SL4120	fla A	SL4049	EMS,χ	—	—	(—)	+	+	(—)		13/20	3/20
fla-566	SL4121	fla A	SL4049	EMS,χ	—	—	(—)	+	+/±‖	(—)		13/20	4/20
fla-569	SL4122	fla C	SL4049	EMS,χ	—	—	(—)	+	+	(—)		13/19	0/20
fla-577	SL4053	fla F	SL4049	EMS,χ	++	—	+	+	+	+	0/5		0/12
fla-586	SL4054	fla F	SL4049	EMS,χ	+	—	+	+	+	+	0/5		0/12
fla-598	SL4055	unique	SL4049	EMS,χ	++	+	+	—	+/—‖	+	4/5		0/11,$>10^{-3}$

fla-604	SL4056	fla F	SL4049	EMS,χ	+	—	+	+	+	+	0/5		0/22
fla-612	SL4057	fla F	SL4049	EMS,χ	+	—	+	+	+	+	0/5		0/12
fla-638	SL4058	unique or fla F?	SL4049	EMS,χ	++	+	+	+	+	+	0/5	0/20	$<10^{-3}$
fla-648	SL4059	fla M	SL4049	EMS,χ	+++	+	+	+	+	+	3/5		$<10^{-3}$
fla-655	SL4060	fla F?	SL4049	EMS,χ	—	—	+	+	+	+	0/5		$<10^{-3}$
fla-676	SL4061	fla B	SL4049	EMS,χ	+	—	+	+	+/±‖	+	5/5		3/18
fla-682	SL4062	fla M	SL4049	EMS,χ	+++	—	+	—	—	n.t.	5/5		$<10^{-3}$
fla-699	SL4063	fla M	SL4049	EMS,χ	+++	+	+	+	+	+	5/5		$<10^{-3}$
fla-702	SL4064	fla F?	SL4049	EMS,χ	+++	+	+	+	+	+	0/5		$<10^{-3}$
fla-711	SL4124	fla F	SL4049	EMS,χ	—	—	(—)	+	+	(—)		0/19	0/20

* EMS, ethyl methane sulfonate; 2AP, 2-aminopurine; NG, nitrosoguanidine; S-S, picked as non-spreading colony in semisolid medium; χ, selected by χ phage.

† *sup-812* and *su7* introduced in F′ factors, *sup-811* and *supD501* by transduction. +, suppressor-bearing derivative more motile than parent. —, no suppression detected. (—), no suppression detected in screening test. n.t., not tested.

‡ Proportion of *his*+ recombinants (in crosses with SL3547 (HfrB2) donor) or of *trp*+ *his*+ recombinants (in crosses with donor SA904) found to be motile.

§ Proportion of motile transductants with *H1* allele, *H1-b*, of donor SL688: $>10^{-3}$ and $<10^{-3}$ indicate positive and negative results when selection was made in semisolid medium with anti-*i* serum, estimated to detect *H1* co-transduction at a frequency of ca. 10^{-3}.

‖ Sets of *supD501* derivatives of the indicated mutants were heterogeneous, comprising some highly motile clones and others which were less motile, in the case of mutants *fla-546*, *fla-566* and *fla-676*, or no more motile than their parent strain, in the case of *fla-598*; the highly motile clones are attributed to cotransduction of *fla*+ with *supD501*.

TABLE 4

Recombination and complementation of nonsense fla mutants and standard fla mutants of complementation groups A, B, C, K and M*

Recipients	Donors, i.e., strain on which phage grown							
	Nonsense *fla* mutants				Standard *fla* mutants			Wild type
Group A								
	fla-401	*fla-526*	*fla-561*	*fla-566*	*flaA41*†	*flaA56*		*fla*+
fla-401	0/0	>4/20	>6/30	>4/30	1/0	>3/5		>4/50
fla-526	>6/13	0/0	>2/7	>4/0	0/0	>5/0		>5/20
fla-561	>5/50	>4/30	1/0	>4/0	0/0	>4/0		>3/15
fla-566	>3/6	0/0	>2/0	0/0	0/0	>4/0		>2/4
flaA41†	4/0	0/0	0/0	0/0	0/0	0/0		>8/15
Group B								
	fla-676				*flaB50*†	*flaB59*	*fla-36*‡	*fla*+
fla-676	1/0				1/0	1/0	0/6	>4/20
flaB50†	>7/1				0/0	1/2	>3/20	>5/11
flaB59	1/3				1/0	0/0	>2/40	>5/65
fla-36‡	>4/90				>5/60	>3/80	1/0	>5/55
Group C								
	fla-546	*fla-569*			*flaC54*†			*fla*+
fla-546	1/0	>6/0			1/0			>4/18
fla-569	3/0	0/0			2/0			>4/26
flaC54†	1/0	3/0			0/0			>1/8
Group K								
	fla-513	*fla-511*			*flaK44*†	*flaK48*		*fla*+
fla-513	0/9	0/0			1/0	0/5		5/25
fla-511	0/0	n.t.			0/0	n.t.		7/25
flaK44†	3/0	3/0			0/0	3/0		10/7
flaK48	n.t.	n.t.			0/0	0/0		3/30
Group M								
	fla-648	*fla-682*	*fla-699*					*fla*+
fla-648	0/0	1/0	2/0					>3/33
fla-682	2/0	0/0	1/0					>5/45
fla-699	>4/0	2/1	0/1					>7/48

* Figures before and after oblique are, respectively, number of swarms and number of trails observed after overnight incubation of standard loopful of transduction mixture on semisolid medium. Data are from tests made to check preliminary assignments. In control mixtures, with broth instead of phage, the leaky mutant *fla-401* spread slowly; all other mutants produced no or at most one swarm. A single "trail" was recorded in the broth control of *flaM699*; no other mutant produced any trails in the broth control.

† These strains, chosen as complementation testers, were crossed with all the nonsense motility mutants.

‡ Strain SJ79, LT2 *fla-36*, was included in these tests because supposedly of *fla* complementation group B, a conclusion not supported by the present data.

the *fla*+ parent), and in stained preparations about the same proportion had flagella, of apparently normal shape and length distribution, but fewer (mean between 1 and 3 flagellate cell) than in *fla*+ strains (mean between 4 and 8). Testing of sets of five or more *supD501* and *sup-811* transductional derivatives of each mutant showed the *supD501* derivatives of four mutants to be heterogeneous, some highly motile and others only moderately motile (in the case of three

mutants) or no more motile than their unsuppressed parent (in the case of one mutant) (Table 3). We attribute the highly motile *supD501* transductant clones to co-transduction of the relevant *fla*$^+$ gene of the donor with its *supD501*, for the affected *fla* loci were all found to be closely linked to *H1* and P22 co-transduction of such *fla* loci and *supD501* is observed (FANKHAUSER and HARTMAN 1971; this paper, Table 2).

Complementation, Hfr mapping and H1 co-transduction: Strains representing known *fla* complementation groups were first intercrossed, as also were strains of *mot* complementation groups A, B and C. The results (P. S. VARY, Ph.D. thesis, Stanford University, 1969) agreed with expectation from earlier work (JOYS and STOCKER 1965; IINO and ENOMOTO 1966; ENOMOTO 1966) except that: (1) *flaE60* and *flaJ28*, the only reported members of these two groups, did not complement each other; (2) *fla-36*, supposedly of group B, complemented the two strains which define this group (and also complemented all the *fla* tester strains used, so that *fla-36* appears to define a complementation group not previously recognized in *S. typhimurium*). Strains chosen as *fla* and *mot* complementation testers (see METHODS) were crossed in each direction with the nonsense motility mutants and 18 of the latter were intercrossed in all possible combinations. These and confirmatory tests (Table 4) showed that four mutants (including the very leaky *flaA401*) fell in *fla* complementation group A, one in group B (defined by *flaB50* and *flaB59*, for the new mutant did complement *fla-36*), two in group C and two in group K. Eight mutants failed to complement the group F tester, *flaF25*, and most intercrosses of these eight gave few or no trails (Table 5); however some pairs complemented well, *e.g.*, *flaF612* with *flaF604*. Three furtsher strains, though they complemented the group F tester, gave no, or few, trails in crosses with some of the eight nonsense mutants assigned to group F; we therefore consider the two complementation groups defined by these three strains (Table 5, foot) to be subgroups of group F. Three mutants complemented all the testers, but not each other (Table 4) and define a new group, M. Mutant *fla-598* complemented all the tester strains and all of 18 other nonsense mutants and thus seems to define a further new group. The flagellate motility mutant, #411, complemented all the *fla* and *mot* testers.

All the nonsense mutants were crossed with one or other of two Hfr donors which transfer the *H1* region and *his* early, and *flaF* (between *gal* and *trp*) late (Table 1), and the donor *his*$^+$, or *his*$^+$ *trp*$^+$, were selected. The results of these tests and of tests of co-transduction of *H1* with *fla*$^+$, etc. genes (Table 3) accorded with expectation (IINO 1969) in the case of mutants assigned to previously known *fla* complementation groups. The nonrepresentation of donor *fla*$^+$ amongst *his*$^+$ recombinants and of donor *H1* amongst *fla*$^+$ transductants supported the notion that the two complementation groups defined by mutants *fla-655*, *fla-702* and *fla638* (Table 5) are sub-groups of Group F. A low frequency of *H1* co-transduction showed that the gene affected in *fla-598*, which defines a new complementation group, is close to *H1*. The representation of donor *fla*$^+$ amongst *his*$^+$ recombinants derived from the three mutants of the new group M suggest that *flaM* also is located in the cluster of motility genes linked to *H1*; but no *H1*

TABLE 5

Recombination and complementation of nonsense fla mutants and standard fla mutants of complementation group F*

Recipients	Donors, i.e., strain on which phage grown — Nonsense *fla* mutants, *fla-*											Tester	Control
fla-	466	530	560	577	586	604	612	711	655†	702†	638†	F25‡	*fla*+
466	0/0	0/0	1/0	>1/13	0/17	0/0	1/10	3/3	>4/23	0/3	3/3	2/0	>2/16
530	0/0	0/0	0/0	>1/2	0/0	4/0	0/0	4/3	>3/2	>1/4	5/14	>4/0	>2/8
560	0/0	0/0	1/0	0/13	0/0	>3/1	0/3	4/0	>3/21	>2/13	3/34	1/0	>9/16
577	0/8	>1/0	>1/6	0/0	0/9	>1/24	0/12	4/0	>4/25	>1/15	4/24	>4/2	>5/18
586	>1/6	>1/0	0/1	>4/12	1/0	>1/22	0/0	>5/12	>2/24	>1/8	1/24	1/0	>7/35
604	0/0	0/0	0/0	1/3	1/3	1/0	1/6	2/18	>1/12	>1/3	2/15	0/1	>5/7
612	0/7	0/0	0/0	0/9	0/0	0/25	1/0	>7/20	>3/23	0/10	2/47	4/0	>4/34
711	0/0	0/0	0/0	0/1	1/2	1/4	1/1	1/0	1/4	0/2	n.t.	1/0	n.t.
655†	2/6	>1/9	1/6	>1/13	3/13	>4/50	1/10	>8/35	1/0	1/0	2/24	>5/30	>5/28
702‡	1/18	0/1	2/4	0/1	1/0	>3/33	>3/10	>5/20	1/0	1/0	1/18	>6/40	>5/25
638†	1/4	0/9	2/10	3/9	0/10	6/41	0/15	n.t.	3/28	0/6	1/0	2/35	6/28
F25‡	1/0	0/0	0/0	1/1	0/0	2/2	0/0	3/0	4/60	>2/15	>3/60	0/0	>3/10

* Figures before and after oblique are, respectively, number of swarms and number of trails seen after overnight incubation of standard loopful of transduction mixture on semisolid medium. Data for crosses with *flaF25* are from complementation screening tests, the rest from an experiment in which most of the nonsense *fla* mutants were crossed with each other.

† Mutants *fla-655* and *fla-702* complemented the group F tester and failed to complement each other; but the complementation group they define is perhaps a sub-group of group F since each of them gave no, or only partial, complementation with some of the new group F mutants. For a similar reason mutant *fla-638* is suspected to belong to group F, even though it complemented the group F tester.

‡ *flaF25*, chosen as a complementation tester, was crossed to all the nonsense motility mutants.

co-transduction (rate $< 10^{-3}$) was detected (Table 3). Tests for co-transduction of *flaM*$^+$ with *his*$^+$ or *trp*$^+$ were also negative (Table 2).

An amber mutation in the structural gene for phase-1 flagellin: The suppressible non-flagellate mutant #521 (SL4045), derived from a *vh2*$^-$ parent fixed in phase 1, complemented all the *fla* testers. Nearly all the motile transductants evoked from it by phage grown on an *H1-b H2-e,n,x* donor expressed antigen *b* (Table 3), which suggested that its mutation was in, or very closely linked to, *H1-i*; one motile transductant showed antigen *e,n,x*, presumably in consequence of the replacement of the "inactive" *H2-e,n,x* allele of the recipient by an "active" *H2-e,n,x* allele from the donor—which suggested that the mutation concerned, unlike a *fla* mutation, prevented formation of phase-1 but not of phase-2 flagella. Tests of the ability of anti-*i* serum to prevent trail formation (see METHODS) showed that the *H1-i* allele of mutant #521 remained unexpressed in motile phase-1 abortive transductants known to carry this gene, whether in endogenote or in exogenote. The mutant failed to complement an *ah1*$^-$ strain (see METHODS) in respect of ability to produce phase-1 flagella. These results showed that the amber mutation of mutant #521 was in *H1*, and we designate it *H1–521*.

Broth cultures of suppressed derivatives of *H1–521* contained 5%–80% of motile cells. Light and electron microscopy showed similar proportions of flagellate bacteria, with flagella which appeared normal in shape and length distribution; the average number of flagella per flagellate bacterium was less than in *fla*$^+$ strains. Sera from rabbits immunized with suppressed derivatives obtained by transduction after complete absorption with wild-type cells still agglutinated the suppressed strains; but the residual antibodies proved to be directed only against somatic factor 1, resulting from P22 conversion.

(In a mating made to introduce *su7* into *H1–521* we encountered a colony of non-motile bacteria which were flagellate, with straight or only slightly curved flagella. This clone (at first mistaken for the F′ *su7* derivative of *H1–521*, VARY and STOCKER, Bact. Proc., 1969, p. 53) was a Pro$^+$ revertant of the *E. coli* donor, LS322. Tests of its ancestors (including W3110, widely used as K12 wild-type) indicated that the straight-flagella mutation occurred in stock culture W2637, prior to 1954 when W3110 was isolated from it (E. M. LEDERBERG, pers. comm.)).

A flagellate but non-spreading mutant, perhaps deficient in chemotaxis: The nonsense motility mutant #411 spread only very slowly in semisolid medium and on microscopy only a few cells showed normal translational motility; but nearly all the rest showed local movement, obviously not merely Brownian. Anti-*i* serum arrested all movement. In a viscous medium most cells traveled, rather slowly, and reversed very frequently, *e.g.*, after less than a second; whereas cells of the motile parent traveled slowly, without frequent reversals. Suppressor genes restored ability to spread rapidly, and in derivatives carrying *supD501* or *sup-811* 60%–80% of cells showed translational motility and the rest abnormal local movements. Electron and light microscopy showed normally flagellate bacteria. In tests of chemotaxis by the capillary tube method (ARMSTRONG, ADLER and DAHL 1967) its parent strain formed a band and mutant #411 did not, but

perhaps only because of its defect in translational motility. Cells of the parent strain located behind the migrating band, and therefore presumably in a depleted region, behaved like mutant cells, i.e., continuously reversed. The affected locus was injected early by HfrB2, but no co-transduction of *H1* with it was detected (rate < 0.02) (Table 3). As we think the mutation concerned causes a defect in chemotactic response (see DISCUSSION), we designate it *che-411*.

DISCUSSION

Our nonsense motility mutants included 23 amber and one ochre *fla* mutants, falling in complementation groups A (4 strains), B (1 strain), C (2 strains), F (8 strains), and two new subgroups of F (3 strains), and K (2 strains); a new group, M (3 strains) and a further new group (1 strain). In addition the amber mutant *fla-36* (FANKHAUSER and HARTMAN 1971) defined a further group. If each of these complementation groups corresponds to a gene there are at least 8 protein-specifying *fla* genes. DEPAMPHILIS and ADLER (1971) have shown the presence in *E. coli* of a complex structure, including four rings and a central rod, at the base of the flagellum, proximal to the hook. Protein-specifying *fla* genes in *S. typhimurium* may be structural genes for proteins constituting the hook and the basal structure components. No amber mutants have been reported in various other *fla* complementation groups, for which the hypothesis of a *fla* gene which specifies only an RNA product is not disproven. The gene defined by the new *fla* complementation group M was not co-transducible with *H1*; deletion mapping of the corresponding gene in *S. abortusequi* indicates the order *H1—motB—flaC-flaM* (YAMAGUCHI *et al.* 1972). The genes affected in *fla-598*, which defined a further new group, was co-transducible with *H1*. Recent crosses of this mutant to *S. abortusequi* deletion mutants indicate the order *flaN—fla-598—flaAIII* (STOCKER and YAMAGUCHI, unpublished data); the gene concerned apparently regulates the length of the hook part of the flagellum, since electron microscopy of this mutant shows structures ("superhooks") which look like abnormally long hooks (DELAFIELD *et al.* 1973).

In general the properties of the nonsense motility mutants corresponded to expectation. However, the first recognized amber mutant, *flaA401* (SL3625) was conspicuously leaky (5%–15% of the bacteria flagellate and motile); a possible explanation would be a mutation near the "distal" end of the gene. Several other amber *fla* mutants were slightly leaky, as shown either by presence of 0.1%–1% of motile cells (5 mutants) or only by production of satellite micro-colonies (6 more mutants). In this sort of leakiness, which is common in *fla* mutants of unknown mutation type (QUADLING and STOCKER 1957), a small fraction of the population manifests a transient change to approximately wild-type phenotype in respect of ability to manufacture flagella. The *fla* deletion mutants of YAMAGUCHI *et al.* (1972) were not noted as leaky, so it seems unlikely that the transient *fla*$^+$ phenotype of many *fla* point mutants results from replacement of the deficient gene product by a product of some other gene. If so then such leakiness in *fla* nonsense mutants must indicate misreading of the nonsense codon as sense

in a few cells; it would be interesting to discover whether such cells also misread other amber codons.

Nonsense mutations, unlike missense mutations at some loci, usually do not complement other mutations of the same complementation group, and it was therefore surprising that several amber mutants of *fla* groups A (Table 4) and F (Table 5) showed intra-group complementation, with some pairs of amber mutants of a single group, e.g., *flaA526* and *flaA561*, complementing each other. Probably the explanation is that each of these two groups corresponds to more than one structural gene—indeed the *flaA41* deletion defining group A is reported to extend over three genes in *S. abortusequi* (YAMAGUCHI *et al.* 1972). Most of the amber *fla* mutants were well suppressed by some or all of the three amber suppressors used but for a few mutants a very weak and variable suppression was revealed only by appearance of a few (0.5%–15%) motile bacteria. We suspect that other weakly suppressible mutants may have gone undetected in our screening tests (and amber *fla* mutants as leaky as *flaA401* would have been discarded during screening).

Two amber motility mutants proved to be of classes other than *fla*. Mutant #521, non-flagellate and derived from an *H1-i* parent fixed in phase 1, complemented all *fla* testers, and its other properties (very high representation of donor *H1* allele amongst motile transductants; failure to complement *ah1*; non-expression of antigen *i* in phase-1 motile abortive transductants carrying the mutant *H1-i* region, in endogenote or in exogenote) indicated mutation in *H1* (or in an adjacent gene controlling its expression). As the mutation is amber we assume it to be in the flagellin structural gene itself, rather than in the *H1*-activator gene postulated by IINO (1961*a*) to account for his *ah1*$^-$ mutants (whose properties, however, now seem compatible with those expected for *H1* nonsense mutants). The flagella of suppressed derivatives of *H1-521* did not differ serologically from wild-type antigen *i*. Either the mutant codon is outside the part of *H1* which determines serological character (known to reside in at most about half the polypeptide, PARISH, WISTAR and ADA 1969) or the wild-type amino acid and those specified by the suppressors are not immunologically determinant.

Cells of the non-spreading mutant, *che-411*, showed anomalous, mainly non-translational movements though they bore morphologically normal flagella. The chemotactic response of bacteria, including *Salmonella*, is thought to be based on the avoidance reaction: that is, increased frequency of reversal or change of direction occurs when an "unfavorable" stimulus is received (WEIBULL 1960). If so, two sorts of general defect in chemotactic behavior might be expected to arise by mutation: failure to give the avoidance reaction even when the environment is "favorable." The chemotaxis-deficient mutants described in *E. coli* correspond to the first class, since they appear normally motile on microscopy (ARMSTRONG, ADLER and DAHL 1967). We think that mutant *che-411* represents the second class of defect, because of the frequent reversals seen when it was examined in a viscous medium and because its movements in broth resembled those of cells of its parent strain located behind a chemotactic band in a capillary tube, and therefore presumably giving an avoidance reaction. R. M. MACNAB

and D. Aswad (personal communication), employing a novel chemotaxis assay (Macnab and Koshland 1972) have recently observed that when *che-411* cells are subjected to a sudden increase ($0 \rightarrow 10^{-3}$M) in the concentration of a chemotactic attractant (L-serine) they achieve smooth translational motility for a short time ($\sim$ 30 sec), then revert to jerky, non-translational motility, whereas the normally motile cells of strain LT2 wild type when so treated show "smoother and better co-ordinated" motility, for up to 5 min. We take this as confirming that the *che-411* mutation affects the mechanism which underlies the chemotactic response. The amber mutation *che-411*, though not co-transducible with *H1*, behaved as an early marker in HfrB2 and the affected locus is presumably (like the *che* loci of *E. coli*, Armstrong and Adler 1969), part of the cluster of motility genes between *trp* and *his*.

We thank our colleagues named above for provision of strains and unpublished information.

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