GENETICS OF MOTILITY IN *ESCHERICHIA COLI*: COMPLEMENTATION OF PARALYZED MUTANTS¹

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ALTHOUGH flagella are regarded as the bacterial motor organs and have been extensively studied, little is still known about the mechanism of their action (for a review see WEIBULL 1960). One approach to an understanding of the movement of flagella has been the study of mutants defective in motility. Non-motile flagellated strains of *Salmonella* have been known for many years (COLQUHOUN and KIRKPATRICK 1932; KAUFMANN 1939; EDWARDS, MORAN and BRUNER 1946; HIRSCH 1947; FRIEWER and LEIFSON 1952). Such variants have generally been referred to as "paralyzed" and those examined in detail were found to have flagella in typical number and shape and to agglutinate with antiserum prepared against wild-type flagella.

Most *Salmonella* can alternate between making one or the other of two kinds of flagella (phase variation), each a polymer of an antigenically different flagellin (McDONOUGH 1965). The two flagellins are determined by different genes, designated *H1* and *H2*, yet paralyzed strains are nonmotile no matter which flagellin is being made. This observation suggests that the mutation affects some factor responsible for the functioning of the flagellum other than flagellin. Genetic studies on *Salmonella* have confirmed this conclusion (MITANI and IINO 1965), and the results reported in this paper show that the same is true of *Escherichia coli*. In addition, we have investigated the number of paralyzed complementation groups and their relationship on the bacterial chromosome. Complementation tests have already led to the classification of paralyzed mutants of *Salmonella typhimurium* into three groups (ENOMOTO 1962, 1966).

General description of the complementation test: In a transduction, the bacteriophage carry small fragments of the donor chromosome to the recipient bacteria. In complete transduction, donor genes transferred by the phage replace their alleles on the recipient genome. STOCKER, ZINDER and LEDERBERG (1953), studying the transduction of flagellar characters by the *Salmonella* phage P22, observed not only swarms, arising from the complete transduction of motility to nonmotile strains, but also trails: linear groups of microcolonies extending out from the site of inoculation (see, for example, Figure 1). A trail marks the path of a bacterium made motile by abortive transduction (STOCKER 1956; LEDERBERG 1956). In such an abortive transduction, the wild-type motility gene introduced by the phage neither recombines with the recipient genome, nor multiplies. It is,

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FIGURE 1.—Transduction of *mot-44* with P1 grown on *mot-46*. The transduction was carried out as described under MATERIALS AND METHODS, and an 0.1 ml aliquot was streaked across a semi-solid tryptone plate. The photograph was taken after 20 hours incubation at 35°C. One swarm, arising from recombination between the alleles, and trails, indicating complementation, are visible. The yield of trails would be scored as ++ (see legend to Table 2).

however, dominant, and capable of determining a wild-type gene product. The bacterium containing the fragment is therefore motile and will swim away from the inoculum, but will leave a trail of colonies of its nonmotile progeny behind it.

The trail phenomenon was applied by IINO (1958) as a complementation test for motility mutants. If the donor and recipient are defective in the same gene, a wild-type gene product can be made only after recombination, and trails can only arise through intragenic complementation. If they are in different genes, and the defective gene products do not interfere with the functioning of the wild-type products, then normal intergenic complementation will occur and trails should be seen.

The complementation tests reported in this paper were carried out using a completely analogous system based on abortive transduction by the phage P1. In *E. coli* abortive transduction by P1 has previously been used to test for complementation of genes of the arabinose pathway (GROSS and ENGLESBERG 1959).

Definition of terms:

1. Flagellum. The term flagellum is used to include the entire organelle, i.e. the structure outside the cell wall plus any attached structure inside.
2. Filament. The long wavy portion of the flagellum extending out from the basal structure.
3. Flagellin. The protein subunit of which the flagellar filament is composed.
4. *H* gene. The gene determining flagellin. *E. coli* is monophasic and therefore has only one *H* gene, whereas *Salmonella* strains usually have two.
5. Curly mutant. A mutant with a filament having a wavelength approximately half that found in the wild type. The alteration results from a mutation in the *H* gene (INO 1962). A curly mutant will not swarm on a semisolid medium. Under the light microscope the bacteria do not show translational movement, but spin.
6. *mot* gene. A gene determining some factor, other than flagellin, responsible for the functioning of the flagellum, a defect in which may lead to paralysis.

MATERIALS AND METHODS

Media: Tryptone broth consists of 1% tryptone (Difco) and 0.5% NaCl. Tryptone plates are made by adding 1% agar (Difco) to tryptone broth. Semisolid tryptone plates contain 0.35% agar. L broth has been described by LENNOX (1955).

Bacteria: Mutants were isolated from three motile strains, AW330, AW405 and AB1884, the properties of which are listed in Table 1. AW330 is a K-12 strain derived from M. L. MORSE's No. 553, which came from the LEDERBERG strain W2580. A motile isolate was picked from the edge of a swarm on a semisolid tryptone plate. AW405 is a derivative of AW330. The *his-4* allele was introduced from strain AT1372 (TAYLOR and THOMAN 1964) by transduction with P1, followed by penicillin selection. AB1884 (HOWARD-FLANDERS, BOYCE and THERIOT 1966) was given to us by DR. HOWARD-FLANDERS, and a motile isolate was obtained from a swarm on a semisolid tryptone plate. It is a K-12 strain, but not directly related to the other two.

Mutant isolation and characterization: A procedure for isolation of paralyzed mutants based on their failure to swarm on a semisolid medium has been described by ARMSTRONG, ADLER and DAHL (1967). Nonchemotactic mutants, nonflagellated mutants, and mutants with morphologically abnormal flagella were also obtained by this procedure. In addition, a number of paralyzed mutants were isolated by selection with the phage chi, which generally attacks only motile strains of *Salmonella* (MEYNELL 1961) and other Enterobacteriaceae (INO and MITANI 1967; SCHADE and ADLER 1967). A colony from a tryptone plate was picked and grown overnight in 1 ml tryptone broth. One-tenth ml was plated on a tryptone plate in 2 ml tryptone 0.35%-agar overlay. A drop of chi phage at 10^8 /ml was spotted on the plate, which was then incubated overnight. An inoculating loop was scraped across the lysed area, and the bacteria were suspended in 1 ml tryptone broth. The suspension was streaked on a tryptone plate, and after incubation, isolated colonies were picked and characterized. The majority of the mutants isolated by this procedure were nonflagellated or paralyzed.

Only one representative of each mutant type was kept from each clone. The procedure for characterizing the mutants was described previously (ARMSTRONG *et al.* 1967).

Procedure for transduction: The P1kc phage, here called P1, was a strain isolated by YANOF-

TABLE 1

*Properties of parental strains**

Strain	<i>lac</i>	<i>gal</i>	<i>ara</i>	<i>xyl</i>	<i>mtl</i>	<i>arg</i>	<i>pro</i>	<i>thr</i>	<i>leu</i>	<i>his</i>	<i>thi</i>	<i>uvrC</i>	T1·T5	T6	<i>str</i>	F
AW330	1	1,2	2	—	+	+	+	—	—	+	—	+	R	S	R	—
AW405	1	1,2	2	—	+	+	+	—	—	4	—	+	R	R	R	—
AB1884	—	—	—	—	—	—	—	—	—	4	—	34	S	R	R	—

* Isolation numbers are given where known. (R) resistant; (S) sensitive; (+) synthesized or utilized; (—) not synthesized or utilized.

SKY and LENNOX (1959) as an especially effective transducing phage and was given to us by DR. YANOFSKY. Stocks of P1 were prepared on each of the donor bacteria by the soft agar layer technique (SWANSTROM and ADAMS 1951). Lysates were titrated on either strain AW330 or AW405 and were stored over chloroform.

The transductions were carried out essentially according to the procedure described by LENNOX (1955). An 0.5 ml sample of a freshly grown L broth culture of the recipient strain, at approximately 2.5×10^8 /ml, was mixed with 0.02 ml of 0.25 CaCl_2 at 35°C. About 4×10^9 infective units of P1 grown on the appropriate donor were added, and the final volume was brought to 2 ml with L broth. After 15 minutes to allow adsorption of the phage, the mixture was centrifuged at $4,300 \times g$ for 15 minutes at room temperature. The bacteria were resuspended in 0.5 ml of 0.15 M NaCl, and 0.1 ml of the resuspended mixture was streaked across a semisolid tryptone plate. In those transductions involving the wild type as donor, 0.1 ml aliquots of 10, 100, and 1000-fold dilutions were also streaked on semisolid plates. Plates were incubated 20 to 24 hours at 35°C in a water-saturated incubator, and then examined for the presence of swarms or trails.

The appearance of swarms (Figure 1) indicated that recombination had occurred between the two mutant alleles, and therefore that they were not identical. The number of swarms was taken as a measure of the proximity of the two mutated sites. If individual swarms could be distinguished, the number was recorded, otherwise the result was scored as confluent.

The appearance of trails (Figure 1) showed that complementation had occurred. The number

TABLE 2

Properties of paralyzed mutants

Mutant No.	Reversion	Trails (wild-type donor)	Mutant No.	Reversion	Trails (wild-type donor)
Group I:			M515	+	++
M204	+	8	M524	—	+
M205	—	+	M531	+	++
M226	—	6	M580†	+	++
M240	+	12	M626	—	++
M448†	—	+	M637	—	+
M473†	+	0	Intermediate:		
M526	—	++	M41	—	++
M564†	+	0	M44	+	++
M566†	+	0	M180	—	+
M567†	—	++	M184	+	+
M570†	—	0	M232	+	++
M619	—	+	M260	+	++
M625	+	2	M284	—	+
M632	+	+	M458†	+	++
M634	+	5	M483†	—	6
M636	+	7	M488	+	+
M639	—	11	M521	+	+
M640	+	7	M525	—	6
M641	+	2	M533	+	*
Group II:			M589	+	+
M46	+	++	M592	+	+
M507	—	++			

* Gives spontaneous trails.

† Mutants isolated after treatment with the mutagen N-methyl-N'-nitro-N-nitrosoguanidine (ADELBERG, MANDEL and CHEN 1965). All others are spontaneous.

M41-46 are derived from AB1884, M180-284 from AW330 and M448-641 from AW405.

The number of trails was recorded if 12 or less. + indicates 13 to approximately 100 trails (determined from a 10-fold dilution) and ++ greater than that number.

of trails was recorded if 12 or less. Above that number the results were scored only on a semi-quantitative basis (see legend to Table 2).

RESULTS

Forty-two independent paralyzed mutants were isolated according to the procedures described above. Each was tested for reversion by streaking a 0.1 ml sample (approximately 10^9 cells) from an overnight tryptone broth culture on a semisolid tryptone plate. The plates were incubated 48 hours at 35°C. The results are listed in Table 2.

In addition to revertant swarms, one of the mutants yielded numerous spontaneous trails in the reversion test, and was not used as a recipient in the complementation tests. Many of the mutants yielded one or two spontaneous trails, but this did not interfere seriously with the complementation tests.

All the paralyzed strains gave swarms, i.e. wild-type recombinants, when transduced with a lysate of a wild-type donor (either AW330 or AW405). The usual yield was in the order of 5×10^{-7} per input phage, as determined from a 100-fold dilution of the transduction mixture. The ratio of abortive to complete transduction, that is trails to swarms, ranged from less than 1:400, the limit of detection, to about 2:1. The yield of trails with the wild-type donor is listed in Table 2.

Complementation between paralyzed mutants: For each mutant a sufficient number of complementation tests were carried out to ensure an unambiguous classification. The results are presented in Figure 2. Responses were grouped into three classes: no complementation (0 to 5 trails), weak complementation (6 to 12 trails) and strong complementation (greater than 12 trails). In complementation tests yielding only a few trails, the trails were generally shorter than in a strong response. Such trails may be spontaneous, as they occasionally occurred in 'selfed' crosses and in the reversion tests.

Those mutants which gave a strong response with a wild-type donor generally gave either a strong or a negative response (at the most only a few trails) with other paralyzed mutants. Those which failed to give a strong response with a wild-type donor have not been included as recipients in Figure 2. When crossed with a paralyzed donor, such strains generally yielded from zero to several dozen trails. These results may simply reflect a normal variation in the response. Results with a single lysate appear to be reproducible within a factor of two, but we have not investigated the possibility that there may be greater variation between lysates. Because of this uncertainty, no inference about complementation can be drawn from a test with a non- or weak-trail-forming recipient. However, in contrast to their behavior as recipients, these strains appear to possess normal trail evoking power when employed as donors. In this respect they are similar to several *flaA* mutants of *Salmonella* reported by JOYS and STOCKER (1965).

Other cases of nonreciprocal complementation are evident in Figure 2. For example, *mot-44* complements strongly with more paralyzed mutants when used as a recipient than it does as a donor, while *mot-180* and *mot-184* show the oppo-

Complementation with a curly mutant: In order to show that none of the mutations leading to paralysis were in the *H* gene, complementation tests were

carried out between representative paralyzed mutants and a curly mutant (M585) isolated from AW405.

A curly mutant does not produce trails when transduced with P1 grown on wild type, presumably because the altered flagellin molecules can interact with the wild-type molecules to produce a flagellum which is still defective. This argument is supported by the observations that abortive transductants arising from a transduction where the donor and recipient possess different flagellar antigens have flagella where both antigens are expressed (PEARCE and STOCKER 1965), and that flagella reconstituted from a mixture of curly and wild-type flagellins are curly (ASAKURA, EGUCHI and IINO 1966). However, this may not preclude the use of a curly mutant as a donor. If the *mot* and *H* genes are different, then many of the gene fragments carrying the wild-type *mot* gene will not carry the defective *H* gene, and trails will be obtained.

The results of the complementation tests using M585 as the donor are listed in Table 3. In every case definite complementation was obtained. The fact that the yield of trails was, in general, about as good as that observed for the wild-type donor may be interpreted as indicating that few of the transducing fragments carry both the *H* and *mot* genes. In addition, every cross produced a high yield of wild-type recombinants (observed as confluent swarming), which would be expected if the genes were not closely linked.

Two-factor crosses: In crosses between paralyzed mutants the number of wild-type recombinants was consistently depressed about 100-fold below the level obtained when a wild-type donor was used, suggesting that the complementation groups may be adjacent. Since each complementation test was, in effect, a two-factor cross, and since all were carried out under essentially identical conditions, a fine structure map may be constructed, based on the assumption that there is a direct relationship between the numbers of recombinants and the distances between mutational sites on the chromosome. Two problems immediately arise. The first is that swarms arising from reversion cannot be distinguished from those arising from recombination. The second is that more than six or eight individual

TABLE 3
Complementation with curly mutant M585

Paralyzed mutant	Complementation group	Trails	
		Wild-type donor	Curly donor
M526	I	++	++
M632	I	+	++
M46	II	++	++
M507	II	++	++
M580	II	++	++
M44	Intermediate	++	++
M180	Intermediate	+	12
M232	Intermediate	++	+
M260	Intermediate	++	+

swarms on a plate cannot be distinguished. The only usable results, therefore, are from those crosses which are inherently subject to the greatest variability.

Data from 171 crosses, involving only those recipients which are relatively stable as judged by their failure to revert in the reversion test, were used in an attempt to construct a map. Because of the inaccuracy of the data, only limited conclusions could be drawn from the results. However, it was clear that the paralyzed mutants studied here are all clustered in a small area of the chromosome, and that the *mot* region may be divided roughly into two halves. One half contains group I mutants and those intermediate types which complement some mutants in group II. The other half contains group II and the intermediates which complement group I mutants. There is very little overlap. However, the data do not permit a more precise statement on the localization of the intermediate mutants.

DISCUSSION

The biochemical nature of the defect, or defects, which cause paralysis is unknown. Since movement in most biological systems utilizes the energy of hydrolysis of the terminal phosphate bond of ATP, it is reasonable to suppose that the same may be true in the bacteria. ENOMOTO (1966) measured ATP and ATPase activity in paralyzed mutants of *Salmonella*. He found that the ATP content and ATPase activity per cell were not significantly different between several paralyzed mutants and the wild type. Isolated flagella of neither wild-type nor paralyzed mutants showed ATPase activity (ENOMOTO 1962).

BEIGHTON, PORTER and STOCKER (1958) have presented evidence that the defect is structural in at least one paralyzed mutant of *Salmonella typhimurium*. In a study of cells isolated after an abortive transduction, they observed some bacteria which were weakly motile, and which they inferred had one functioning and several nonfunctioning flagella. A functioning flagellum may therefore be transmitted as a unit in a paralyzed strain. The defective structure cannot be the flagellar filament, either from the genetic evidence (MITANI and IINO 1965; this communication), or from the observations that shape and serology are normal and that paralysis is not phase-specific in *Salmonella*. In addition, isolated flagellar filaments have been studied by physical chemical methods, and do not differ from wild type (BEIGHTON *et al.* 1958).

The present work indicates that mutations causing flagellar paralysis are localized in a small region of the *E. coli* chromosome distinct from the *H* gene, but does not clearly indicate the number of genes involved. Ultimately the complementation pattern will probably be explained in terms of one of two alternate models. Either complementation in the *mot* region will turn out to be intragenic, resulting from interactions between identical subunits mutated at nonidentical sites (CRICK and ORGEL 1964), or it will be found to be intergenic. In the latter case specific instances of weak complementation where strong is expected might be explained if there is interaction between nonidentical subunits. One of the best criteria for intragenic complementation is the demonstration that the protein formed by complementation is abnormal, and that no wild-type product can be

found (PERRIN 1963; SCHLESINGER and LEVINthal 1963). However, as our present state of knowledge includes no information indicating the number of proteins defined by the *mot* region, the issue remains in doubt.

Another problem requiring explanation is nonreciprocal complementation. At least two hypotheses, which are not mutually exclusive, may be proposed, but again a definitive answer cannot be made without a knowledge of gene products. The first is that it could be, in part, a gene dosage effect. The fragment introduced by the transducing phage constitutes only one gene copy, while the recipient growing exponentially on L broth probably contains two to four chromosomes. If a critical ratio of subunits is required for an active protein, changing the ratio will alter the response. The second is that complementation may be inhibited if defective subunits are incorporated into a structural component of the cell, and the defective structure cannot easily be corrected without being totally replaced.

The mapping and complementation results are similar to those obtained by ENOMOTO (1966) in *Salmonella typhimurium*. The differences appear to be ones largely of classification and presentation. ENOMOTO's results are organized on the basis of percentage of the response obtained with a wild-type donor, rather than on a basis of the absolute number of trails. Although he has divided the *mot* region into *motA* and *motB* cistrons, he suggests that the existence of "weak-complementing mutants" may be interpreted as evidence for a single structural gene. Non-reciprocally complementing pairs (for example *motA297* : *motB225*) were also encountered.

A third functional unit, corresponding to ENOMOTO's *motC*, was not found, but this may not reflect any major difference between the organisms. Since ENOMOTO found only three *motC* mutants in a total of 97, the total of 42 *E. coli* mutants examined in this work may have been too small to include a *motC* mutant, if they exist.

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SUMMARY

Complementation tests based on abortive transduction with the phage P1 have been carried out between 42 paralyzed mutants of *Escherichia coli*. Although the results do not clearly divide the mutants into cistrons, they do indicate the existence of two groups. Intermediate types, weak complementation, and non-reciprocal complementation are common. The pattern might be attributed either to interaction between the products of two different genes or to extensive intragenic complementation. The results are similar to those obtained by ENOMOTO (1966) in *Salmonella*, and in both cases the *mot* region is clearly distinct from the *H*-gene.

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