Transductional Analysis of Non-motile Mutants in *Proteus mirabilis*

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SUMMARY

Twenty-six non-flagellate and two paralysed mutants of *Proteus mirabilis* strain 13 were investigated by transduction with phage 34.13. Abortive transduction divided *fla* mutants into eight complementation groups (*flaA*, *B*, *C*, *D*, *E*, *H*, *J*, *K*) while the *mot* mutants formed one group. Intracistronic and non-reciprocal complementation was found in certain complementation groups. With the exception of *flaH*, which could not be mapped, all *fla* genes were linked to each other and to the *mot* gene. A genetic map of *fla* and *mot* mutations was constructed. Representatives of the eight *fla* mutant groups did not elicit flagellar antibodies in rabbits and are probably unable to synthesize flagellin.

INTRODUCTION

Organisms may be rendered non-motile by mutations which inhibit flagellar synthesis or affect the function of morphologically normal flagella (Stocker, Zinder & Lederberg, 1953). Genes *H1* and *H2* code for phase 1 and 2 flagellin respectively (Iino, 1969); other genes which code for flagellar formation are known as *fla* while those which code for motility are called *mot*.

In *Salmonella typhimurium* *fla* mutants have been divided into nine complementation groups: *flaA*, *B*, *C*, *D*, *E*, *F*, *J*, *K* and *M* (Iino & Enomoto, 1966; Iino, 1969; Vary & Stocker, 1969). *FlaE* and *flaJ* probably belong to the same group (Yamaguchi, Iino, Horiguchi & Ohta, 1972). Thirteen *fla* complementation groups have been described in *Salmonella abortusequi*: *flaA*, *B*, *C*, *D*, *E*, *F*, *G*, *K*, *L*, *M*, *N*, *P* and *Q* (Iino, 1969; Yamaguchi *et al.* 1972). With the exception of *flaF*, *G* and *M* all the *fla* mutants in both organisms are linked to *H1*. The latter gene controls the serological character of phase 1 flagella (Iino, 1969). *FlaF* maps between *gal* and *try* in another part of the chromosome (Smith & Stocker, 1962) while *flaM* is located near *H1* but is not co-transducible with it by phage P22 (P. S. Vary & B. A. D. Stocker, personal communication in Yamaguchi *et al.* 1972). *FlaG* has not yet been mapped (Yamaguchi *et al.* 1972). Enomoto (1966*a*, *b*) and Yamaguchi *et al.* (1972) have grouped paralysed mutants of *S. typhimurium* and *S. abortusequi* into three cistrons, *motA*, *B* and *C*. The groups are linked to *H1* (Yamaguchi *et al.* 1972). Aflagellate mutants have also been demonstrated in *Escherichia coli* (Furness, 1958; Ørskov & Ørskov, 1962; Matsumoto & Tazaki, 1967). These genes are located in at least three regions on the *E. coli* chromosome (see Armstrong & Adler, 1969). Armstrong & Adler (1967) provisionally divided paralysed mutants of *E. coli* into two complementation groups which may correspond to *motA* and *B* in *Salmonella* (Iino, 1969). In *Bacillus subtilis* three unlinked genes (*fla*, *H* and *mot*) which control motility have been described (Stocker, 1963; Joys & Frankel, 1967).

Table 1. *Properties of non-motile mutants*

Mutant	Spontaneous trails	Previous designation	Reference
* <i>flaA1</i> †	+	FM4 <i>fla</i>	Appelbaum <i>et al.</i> (1971)
<i>flaA2</i>	—	FM5 <i>fla</i>	
<i>flaA3</i>	—	FM8 <i>fla</i>	
<i>flaA4</i>	—	<i>fla1</i>	Appelbaum <i>et al.</i> (1972)
<i>flaA5</i>	—	<i>fla2</i>	
<i>flaA6</i>	—	<i>fla3</i>	
<i>flaA7</i>	—	<i>fla4</i>	
<i>flaA8</i>	—	<i>fla5</i>	
<i>flaA9</i>	—	<i>fla6</i>	
<i>flaA10</i>	—	<i>fla7</i>	
* <i>flaB1</i> †	—	<i>fla8</i>	
<i>flaB2</i>	—	<i>fla9</i>	
<i>flaB3</i>	—	<i>fla10</i>	
<i>flaC1</i>	—	.	Unpublished
<i>flaC2</i>	—	.	
* <i>flaC3</i> †	—	<i>fla11</i>	Appelbaum <i>et al.</i> (1972)
* <i>flaD1</i> †	—	<i>fla12</i>	
<i>flaD2</i>	—	<i>fla13</i>	
<i>flaD3</i>	—	.	Unpublished
* <i>flaE1</i> †	+	.	
<i>flaE2</i>	—	.	
<i>flaE3</i>	—	.	
<i>flaE4</i>	—	.	
* <i>flaJ1</i> †	+	.	
* <i>flaK1</i> †	—	.	
<i>flaH1</i> †	—	.	
<i>mot-1</i>	—	FM3 <i>mot</i>	Appelbaum <i>et al.</i> (1971)
* <i>mot-2</i>	—	FM18 <i>mot</i>	Appelbaum <i>et al.</i> (1971)

* Mutants used in linkage experiments with auxotrophic markers and *str*.

† Mutants tested for production of flagellar antibodies.

An outstanding characteristic of most members of the *Proteus* group is their swarming ability (Hauser, 1885; Russ-Münzer, 1935). Flagella probably play a major role in swarming. *Proteus* 'O' variants which may represent aflagellate mutants were first described by Weil & Felix (1917). Coetzee (1963) transduced the swarming property into non-swarming variants of *Proteus mirabilis*. Separate loci which control flagellar synthesis and swarming were demonstrated. The former group was probably *fla* and the latter group, which may correspond to *che* mutants in *Salmonella* (Armstrong, Adler & Dahl, 1967) was found to represent at least three sites (Coetzee, 1963).

Abortive transduction of motility (Lederberg, 1956; Stocker, 1956) has been demonstrated in all members of the *Proteus*-*Providencia* group (Appelbaum *et al.* 1972). This paper reports results of complementation and linkage tests with *fla* and *mot* mutants of *Proteus mirabilis*. Knowledge of the motility systems of this organism could be important for resolution of problems related to swarming.

METHODS

Bacteria. Twenty-six *fla* mutants and two *mot* mutants of *Proteus mirabilis* strain 13 (Coetzee & Sacks, 1960a) were used (Table 1). Isolation and characterization were as

described (Appelbaum *et al.* 1972) and hanging-drop preparations were also used. Mutants *flaD2* and *flaE3* were isolated by serial broth culture of strain 13 in the presence of the flagellar phage χ_7 (Appelbaum, Hugo & Coetzee, 1971). Phage χ_7 -resistant colonies could not be detected, as background growth on agar was too dense. Serial passage of the wild-type organism through phage χ_7 in broth yielded few *fla* mutants and *mot* mutants were not found. The *mot* mutants agglutinate to titre with wild-type antiserum and show normal flagellation, with respect to both the number of flagellated cells and the number of flagella per cell. All non-motile mutants were resistant to phage χ_7 (Appelbaum *et al.* 1971) as are corresponding mutants of *Salmonella* and *Escherichia coli* to phage χ (Iino, 1969). The 75 strains of the Kauffmann-Perch collection of *Proteus hauseri* were obtained from Professor F. Kauffmann and Dr I. Ørskov (Statens Seruminstitut, Copenhagen). Streptomycin-resistant (*str-r*) mutants were isolated according to the method of Coetzee & Sacks (1960a). Organisms with mutations in the following genes were used in linkage experiments: *arg* (Prozesky, 1968a), *met* (Grabow, 1972a), *cys* (Grabow, 1972b), *try* (Grabow, 1970), *his*, *tyr*, *ilv*, *pur*, *pro*, *pyr*, *thr*, *ser* and *leu* (Prozesky, 1968b). Urease deficient (*ure*) mutants were isolated by Coetzee & Prozesky (unpublished). Strains were maintained on agar slopes at 4 °C. Incubation temperature was 30 °C.

Media. Semisolid medium was that of Appelbaum *et al.* (1972). Difco MacConkey agar with 1 mg streptomycin sulphate/ml was used for selection of the *str* marker (Coetzee & Sacks, 1960a). The minimal medium (Grabow & Smit, 1967; Prozesky, 1967) and broth (Coetzee & Sacks, 1960b) have been described.

Transduction. Lysates of phage 34.13 (Coetzee & Sacks, 1960a) with plaque-forming titres of 5×10^9 to 1×10^{10} /ml were prepared on *str-r*, *fla str-r* and *mot str-r* donors as described before (Prozesky, 1968a). In complementation and recombination tests 1 ml of an overnight broth culture of recipient bacteria (about 3×10^8 /ml) was mixed with phage lysates at an input multiplicity of 10. After adsorption for 15 min bacteria were centrifuged and the pellets resuspended in 1 ml of 0.9 % (w/v) NaCl. Samples (0.5 ml) of the transduction mixture were plated for scoring of streptomycin resistance according to the method of Coetzee & Sacks (1960a). The remainder was diluted 1/10 in 0.9 % (w/v) NaCl and 0.5 ml of this suspension streaked across semisolid agar plates in approximately 0.025 ml samples. Linkage with auxotrophic markers was tested by the method of Enomoto (1966a). Approximately 100 swarms from crosses between recipients representing each *fla* and *mot* cistron and auxotrophic donors were scored for auxotrophy. In crosses with *ure* mutants swarms were scored for urease activity according to the method of Singer (1950). Linkage with *str* was tested by use of *fla str-r* and *mot str-r* donors with the wild-type recipient. Scoring was for *str-r* non-motile transductants. Controls were as described (Appelbaum *et al.* 1972). Trails were scored semiquantitatively according to the method of Iino & Enomoto (1966). Trail production (which reflects complementation) with wild-type phage was regarded as grade 3; grade 0 represented no trails, grade 1 1 % to 10 % of control, grade 2 10 % to 50 %, and grade 3 50 % to 100 % of the control. Genetic distances were calculated by expressing the number of swarms (i.e. recombinant transductants) obtained in crosses with phage prepared on *fla* and *mot* mutants as a percentage of the number of swarms obtained with wild-type phage (Enomoto, 1966a). Distances were normalized with regard to donor and recipient capacities by means of a ratio test with the unlinked *str-r* marker (Prozesky, 1968a).

Serology. Two rabbits were immunized with one *fla* mutant from each group according to the method of Iino & Enomoto (1966). Serological techniques were those of Kauffmann (1951).

H-antigenic mutants. The method of Joys & Stocker (1966) was used.

Table 2. Complementation between *fla* and *mot* mutants

Recip- ient	Donor																										wild- type		
	<i>flaA1</i>	<i>flaA2</i>	<i>flaA3</i>	<i>flaA4</i>	<i>flaA5</i>	<i>flaA6</i>	<i>flaA7</i>	<i>flaA8</i>	<i>flaA9</i>	<i>flaA10</i>	<i>flaB1</i>	<i>flaB2</i>	<i>flaB3</i>	<i>flaC1</i>	<i>flaC2</i>	<i>flaC3</i>	<i>flaD1</i>	<i>flaD2</i>	<i>flaD3</i>	<i>flaE1</i>	<i>flaE2</i>	<i>flaE3</i>	<i>flaE4</i>	<i>flaJ1</i>	<i>flaK1</i>	<i>flaH1</i>		<i>mot-1</i>	<i>mot-2</i>
<i>flaA1</i>	S(o)	S(o)	S(o)	S(o)	S(o)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA2</i>	S(o)	S(o)	S(o)	S(o)	S(o)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA3</i>	S(o)	S(o)	S(o)	S(o)	S(o)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA4</i>	S(o)	S(o)	S(o)	S(o)	S(o)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA5</i>	S(o)	S(o)	S(o)	S(o)	S(o)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA6</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(o)	S(o)	S(o)	S(o)	S(o)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA7</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(o)	S(o)	S(o)	S(o)	S(o)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA8</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA9</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaA10</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaB1</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaB2</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaB3</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaC1</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaC2</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaC3</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaD1</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaD2</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaD3</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaE1</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaE2</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaE3</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaE4</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaJ1</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaK1</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
<i>flaH1</i>	O(o)	O(o)	O(o)	O(o)	O(o)	O(o)	O(o)	O(o)	O(o)	O(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	C(o)	O(o)	O(o)
<i>mot-1</i>	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)	S(o)
<i>mot-2</i>	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(1)	S(1)

S = swarm production. Numbers in parenthesis indicate degree of trail production as a % of those evoked with wild-type donor organism: o = no trails; 1 = 1% to 10%; 3 = 50% to 100%.

Table 3. *Reciprocal transduction between mutants*

Recipient	Donor									
	<i>flaA</i>	<i>flaB</i>	<i>flaC</i>	<i>flaD</i>	<i>flaE</i>	<i>flaJ</i>	<i>flaK</i>	<i>mot-1</i>	<i>mot-2</i>	
<i>mot-1</i>	*74.20-77.43	14.38-15.14	83.42-84.49	63.33-66.89	51.38-53.78	54.61-56.32	10.60-12.34	0	1.06-2.93	
<i>mot-2</i>	73.80-77.68	12.54-13.77	85.47-86.63	63.25-65.69	49.33-52.49	54.21-56.11	10.37-13.49	1.01-2.46	0	
<i>flaA</i>	1.17-3.16	59.03-63.51	10.38-12.81	10.15-14.46	22.26-26.88	18.68-21.87	63.15-66.88	72.23-76.93	73.18-77.35	
<i>flaB</i>	59.46-63.59	1.67-2.91	70.66-72.58	49.18-52.59	33.98-36.33	40.15-42.23	4.91-5.57	12.11-15.98	13.11-14.74	
<i>flaC</i>	10.02-12.82	70.13-72.32	1.81-2.70	19.58-22.23	34.93-36.68	29.16-32.72	74.92-76.08	84.14-86.64	84.95-86.31	
<i>flaD</i>	10.03-12.98	49.68-52.54	19.88-22.77	1.36-2.52	12.45-16.17	10.15-11.58	56.27-57.79	63.86-65.79	65.66-66.90	
<i>flaE</i>	23.54-26.58	33.25-36.62	33.89-36.63	14.13-16.96	1.39-2.44	4.74-5.86	39.91-41.72	52.76-53.23	50.34-52.29	
<i>flaJ</i>	18.90-22.11	40.77-41.45	29.52-31.34	10.47-10.84	4.37-6.01	0	44.41-46.05	52.17-55.90	53.13-57.14	
<i>flaK</i>	63.32-66.42	5.59-5.89	71.95-76.21	53.70-57.09	38.90-42.17	42.18-45.06	0	9.18-10.11	9.25-11.64	

* Figures are the range (%) obtained with mutants in each cistron when number of swarms were divided by number of swarms yielded with wild-type donor. Factors to correct for variant recipient and donor capacities were obtained from the same cross with selection for *str-r*. Number of revertants was subtracted. Values are the mean of two experiments.

Fig. 1. Arrangement of motility genes in *Proteus mirabilis*. *Fla*, genes determining presence of flagella; *mot*, genes determining function of flagella. Map is not to scale. The position of *mot* and *fla* genes on the *P. mirabilis* chromosome has not been established.

RESULTS

Complementation tests. 'Selfed' crosses yielded trails on occasions. This has been encountered previously (Appelbaum *et al.* 1972). Transductions from the wild-type to all *fla* mutants except *flaH1* and *mot-1* yielded swarms and trails. No trails were obtained from *mot-1* and no transductants were encountered from *flaH1* even with inocula from undiluted transduction mixtures. Transductions were done in pairwise combinations of *fla* and *mot* mutants (Table 2). The *fla* mutants were subdivided into eight groups and the two *mot* mutants formed one group. Intracistronic and non-reciprocal complementation (Fincham, 1966; Iino & Enomoto, 1966; Armstrong & Adler, 1967) was observed between some mutants in the same group in the case of *flaA*, *flaE* and *mot*. Intracistronic complementation yielded trails which were often shorter than normal. Mutants *flaA9*, *flaC1*, *flaC2* and *flaE4* were poor recipients but were normal donors. No trails or swarms were obtained from *flaH1* while numerous trails and few swarms were seen when this mutant was used as donor. As recipient *mot-1* did not yield trails but numerous swarms and trails were seen with it as donor. Crosses between *mot* and *fla* organisms yielded grade 3 (normal) complementation except with *flaA9*, *flaC1*, *flaC2*, *flaE4* and *mot-1* as recipients.

Recombination. Transductions were done between pairwise combinations of *fla* and *mot* mutants (with the exception of *flaH*) (Table 3). Distances were calculated and normalized as described in Methods. Mutants in the same *fla* cistron are closely linked and there are various degrees of linkage between mutants in different cistrons. Because of their poor recipient ability *flaA9*, *flaC1*, *flaC2* and *flaE4* could only be mapped as donors. Because of its behaviour in crosses *flaH1* could not be mapped.

Linkage experiments with *mot-1* and *mot-2* as markers. The two *mot* mutants are closely linked. Transductions were performed between all *fla* mutants (except *flaH1*) and the two *mot* mutants (Table 3). Linkage was demonstrated between all the *fla* cistrons and the *mot* locus. Results corresponded with those obtained from crosses between *fla* mutants. A linkage map of *fla* and *mot* mutants was constructed (Fig. 1).

Linkage experiments with auxotrophic and *str-r* markers. Linkage could not be demonstrated to any of these markers.

Antigenicity of non-flagellate mutants. Antisera against mutants from all eight cistrons showed no agglutination with H antigen from the wild-type or *mot* organisms.

H-antigenic variants. No serological mutants (Joys & Stocker, 1966) were isolated. Phage 34.13 does not plate on H-serological variants of the Kauffmann-Perch collection. These variants could therefore not be used in genetic studies.

DISCUSSION

The genes concerned with the formation and function of flagella in *Proteus mirabilis* fall into two groups (*fla*, *mot*) as in other organisms studied (Joys & Frankel, 1967; Armstrong & Adler, 1969; Iino, 1969). With the exception of cistrons *flaG*, *L*, *N*, *P*, *Q* which have only

been described in *Salmonella abortusequi*, the arrangement of *fla* genes in *S. typhimurium* and *S. abortusequi* is similar (Iino, 1969; Yamaguchi *et al.* 1972). Although *flaN*, *P*, *Q* were not found in *S. typhimurium* results of complementation tests suggest that these cistrons may be present in both organisms (Yamaguchi *et al.* 1972). The arrangement of *mot* loci is similar in the two organisms (Enomoto 1966*a, b*; Yamaguchi *et al.* 1972). *Proteus mirabilis* differs in having eight *fla* cistrons and one *mot* cistron. As phase variation is a characteristic of *Salmonella* and the swarming phenomenon is typical of *P. mirabilis* the differences in arrangement of *fla* and *mot* genes may reflect different motility-controlling mechanisms in these organisms.

No mutant corresponding to the *flaF* (Smith & Stocker, 1962) or *flaM* (Vary & Stocker, 1969) groups was isolated. The *flaH* mutant encountered in these experiments differs from the *flaG* mutant of Iino & Enomoto (1966) in its poor yield of swarms when used as donor and its failure to elicit flagellar antibodies in rabbits. Joys & Stocker (1965) have described *fla* mutants which, like *mot-1*, yield no trails in transduction even with wild-type phage. They explained this phenomenon by postulating the presence of an unstable modifier gene which causes poor development of flagella in abortive transductants.

An *H* gene in *Proteus mirabilis* was not identified. This was due to failure to isolate serologically different mutants and the resistance of all the Kauffmann-Perch strains to phage 34.13. Joys & Stocker (1966) failed to isolate H-antigenic mutants by selection with some phase I antisera but were successful with other phase I sera.

Enomoto (1967) has described a method of isolating *mot fla* double mutants based on a difference in the colonial morphology of the two groups. This 'dimorphism' (Enomoto & Iino, 1963) was not encountered in our system.

Many mutants in the same cistron showed intracistronic or non-reciprocal complementation. This phenomenon was also encountered in *Salmonella* (Iino & Enomoto, 1966; Yamaguchi *et al.* 1972) and *Escherichia coli* (Armstrong & Adler, 1967). The number of trails and swarms formed in intracistronic crosses was so small that this did not interfere with linkage analysis. Trails occasionally encountered in reversion tests and 'selfed' controls have been described in other systems (Quadling & Stocker, 1957; Armstrong & Adler, 1967). They are presumed to have arisen spontaneously from non-motile mutants.

Failure of *fla* mutants to elicit flagellar antibodies in rabbits may indicate that they are blocked at the level of flagellin synthesis.

No explanation can be given for the few *mot* mutants isolated despite screening of many colonies after mutagenization.

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