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GENETIC MAPPING OF GENES REQUIRED FOR MOTILITY IN *CAULOBACTER CRESCENTUS*

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ABSTRACT

Mutations in more than 30 genes affect motility in *Caulobacter crescentus*. We have determined the chromosomal map locations for 27 genes involved in flagellar morphogenesis (*fla*), three genes involved in flagellar function (*mot*), and three genes that have a pleiotropic effect on both motility and bacteriophage resistance (*ple*). Three multigene clusters have been detected at widely separated chromosomal locations, but in addition, there are 12 *fla* and *mot* genes that are found at eight additional sites scattered around the *C. cresentus* chromosome. Thus, there is more scatter of genes involved in flagellar structure and function than has been observed in other bacterial systems.

CAULOBACTER crescentus is an unusual bacterium in that it undergoes morphogenesis during its normal logarithmic growth cycle (POINDEXTER 1964). Two cell types, a stalked cell and a motile swarmer cell, are produced at each cell division. The stalked cell immediately begins a new cell cycle, whereas the swarmer cell must lose its flagellum and synthesize a stalk before beginning to replicate its chromosome (SHAPIRO 1976). As chromosome replication nears completion, the predivisional cell synthesizes the flagellum, pili and polar phage receptors which are characteristic of the daughter swarmer cell. Thus, *C. crescentus* has a cell cycle that is characterized by two distinct periods of morphogenesis.

During the last several years, our laboratory has worked to develop a system for the genetic analysis of *C. crescentus*. We have developed techniques for the isolation of mutants (JOHNSON and ELY 1977, 1979; ELY and CROFT 1982), for generalized transduction (ELY and JOHNSON 1977), and for RP4-mediated conjugation (ELY 1979) and have used these techniques to construct a genetic map for *C. crescentus* (BARRETT *et al.*, 1982a,b). These developments have led to the potential to perform detailed genetic analyses of mutations affecting any particular trait.

We have chosen to analyze mutations affecting flagellum morphogenesis and function in *C. crescentus*. Since this process occurs at a precise time in the cell cycle, mutations affecting the temporal expression of the *fla* genes should be obtained in addition to those affecting structural proteins and those involved in assembly of the flagellar organelle. JOHNSON and ELY (1979) had isolated a collection of 69 motility mutants that were unable to swim. Of these mutants

seven had a nonfunctional but morphologically normal flagellum (*mot* mutants), and the remainder had no filament or only a stub of a filament (*fla* mutants). A rudimentary transductional analysis of these mutants suggested that the mutations in these strains were located in 29 separate transductional linkage groups. In addition, we isolated mutants that were selected for resistance to bacteriophage φCbK and had simultaneously become nonmotile. Previous work in our laboratory (M. REIBMAN and B. ELY, unpublished results) and by FUKUDA *et al.* (1981) has shown that these pleotropic (*ple*) mutants contain mutations in any of three linkage groups. Mutations in two of these linkage groups, *pleA* and *pleB*, result in cells lacking a flagellar filament. In contrast, *pleC* mutants have a paralyzed flagellum and lack a stalk except under conditions of phosphate limitation. In this paper, we present a more comprehensive analysis of all of these mutants and show the chromosomal location of each mutation.

MATERIALS AND METHODS

Bacterial strains: The *fla* and *mot* mutants used in this study were isolated by JOHNSON and ELY (1979). Spontaneous rifampin- or streptomycin-resistant mutants were used as recipients in conjugation experiments. Additional strains used in this study are described in Table 1. The *ple* mutants were detected by screening phage-resistant mutants for concomitant loss of motility. Growth media have been described by JOHNSON and ELY (1977). Conjugation experiments with derivatives of RP4 were performed as described by ELY (1979) and transductions were performed using φCr30 (ELY and JOHNSON 1977). Values in the text represent the average of two or more independent determinations. Except in preliminary experiments, approximately 200 recombinants were analyzed for the presence of unselected markers in each determination.

RESULTS

General strategy for mapping fla *mutations*: JOHNSON and ELY (1979) identified 26 *fla* genes by transductional analysis of 60 nonmotile mutants. To determine the approximate map location of these *fla* mutations, we transduced an auxotrophic mutation resulting from an insertion of Tn*5* (ELY and CROFT 1982) into a strain containing a *fla* mutation and then introduced pVS1, a kanamycin-sensitive derivative of RP4 (BARRETT *et al.* 1982a), by conjugation. The resulting strains were used as donors in conjugational crosses with auxotrophic mutants as recipients. Mutants were chosen to represent various locations on the *C. crescentus* genetic map (BARRETT *et al.* 1982a) so that linkage to any region of the chromosome could be detected. The recipient strains also contained an antibiotic resistance marker (streptomycin or rifampin resistance) so that selection for both prototrophy and antibiotic resistance provided a double selection against the donor strain. Bacteria from prototrophic colonies appearing on the selective plates were screened for motility in order to determine whether the *fla* marker was coinherited with the selected marker. Alternatively, strains containing a *fla* mutation were used as recipients in crosses with donor strains containing Tn*5* insertions. A series of such donors was described by BARRETT *et al.* (1982a). In these crosses, the kanamycin resistance of the donor was the selected marker and recombinant colonies were screened for the concomitant loss of the *fla* mutation. Similar experiments were performed with

TABLE 1

Bacterial strains

Strain	Genotype	Derivation or Source
CB15	wild type	POINDEXTER (1964)
SC117	*ilvB101*	BARRETT *et al.* (1982b)
SC126	*aux*	BARRETT *et al.* (1982a)
SC141	*metD104*	BARRETT *et al.* (1982a)
SC296	*pleA101*	JOHNSON and ELY (1977)
SC383	*leuA109*	Spontaneous in CB15
SC392	*proA103*	BARRETT *et al.* (1982b)
SC393	*aroG109*	Spontaneous in CB15
SC419	*pleA150*	Spontaneous in CB15
SC420	*aroF110*	Spontaneous in CB15
SC436	*cysC104*	BARRETT *et al.* (1982b)
SC458	*hisD117*	BARRETT *et al.* (1982b)
SC459	*metB115*	BARRETT *et al.* (1982b)
SC526	*pleA102*	Spontaneous in CB15
SC527	*pleA103*	Spontaneous in CB15
SC529	*pleA105*	Spontaneous in CB15
SC533	*pleB109*	Spontaneous in CB15
SC603	*pleB132*	Spontaneous in CB15
SC604	*pleB133*	Spontaneous in CB15
SC605	*pleB134*	Spontaneous in CB15
SC606	*pleC135*	Spontaneous in CB15
SC608	*pleA137*	Spontaneous in CB15
SC609	*pleA138*	Spontaneous in CB15
SC610	*pleC139*	Spontaneous in CB15
SC611	*pleA140*	Spontaneous in CB15
SC613	*pleB142*	Spontaneous in CB15
SC614	*pleA143*	Spontaneous in CB15
SC615	*pleC144*	Spontaneous in CB15
SC616	*pleA145*	Spontaneous in CB15
SC617	*pleC146*	Spontaneous in CB15
SC618	*pleC147*	Spontaneous in CB15
SC874	*metB123::*Tn*5 proA103 str-140*	ELY and CROFT (1982)
SC932	*leuA131::*Tn*5 proA103 str-140*	ELY and CROFT (1982)
SC1084	*trpC109::*Tn*5 str-152*	ELY and CROFT (1982)
SC1091	*cysD137::*Tn*5 str-152*	ELY and CROFT (1982)
SC1288	*hisB137::*Tn*5 ts-104 str-153* (pVS1)	BARRETT *et al.* (1982a)
SC1293	*trpC107::*Tn*5 ts-104 str-153* (pVS1)	BARRETT *et al.* (1982a)
SC1300	*pheA108::*Tn*5 ts-104 str-153* (pVS1)	BARRETT *et al.* (1982a)
SC1321	*metF127::*Tn*5 ts-104 str-153* (pVS1)	BARRETT *et al.* (1982a)
SC1381	*cysB135::*Tn*5 ts-104 str-153* (pVS1)	BARRETT *et al.* (1982a)
SC1388	*aux rif-148*	BARRETT *et al.* (1982a)
SC1417	*serA113::*Tn*5 ts-104 str-153* (pVS1)	BARRETT *et al.* (1982a)
SC1489	*cysE103 rif-176*	Rif in SC415
SC1490	*leuA131::*Tn*5 ts-104 str-153* (pVS1)	BARRETT *et al.* (1982a)
SC1491	*cysE140::*Tn*5 ts-104, str-153* (pVS1)	BARRETT *et al.* (1982a)
SC1582	*hunA106::*Tn*5 str-152*	D. M. FERBER and B. ELY, unpublished
SC1585	*hunB109::*Tn*5 str-152*	D. M. FERBER and B. ELY, unpublished
SC1588	*hunE112::*Tn*5 str-152*	D. M. FERBER and B. ELY, unpublished
SC1591	*hunC115::*Tn*5 str-152*	D. M. FERBER and B. ELY, unpublished

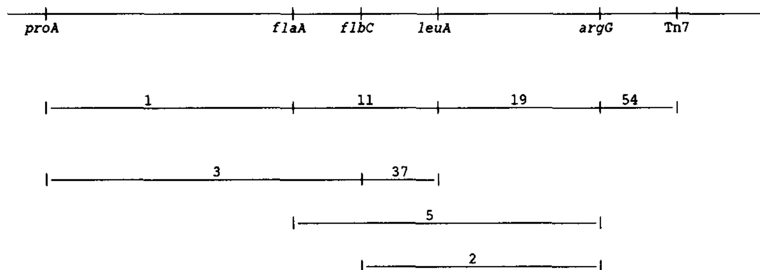

FIGURE 1.—Transductional mapping of *flaA* and *flbC*. Numbers represent percent cotransduction.

the three *mot* genes and the three *ple* genes previously identified (JOHNSON and ELY 1979; M. REIBMAN and B. ELY unpublished data). For each mutation, the observed conjugational linkage formed the basis for more precise mapping experiments as will be described.

Precise location of the flaA *gene*: Since the *flaA* mutation was shown by conjugation experiments to lie in the vicinity of *argG*, phage grown SC229 (*flaA104*) were used to transduce strains containing *argG* or *leuA* mutations to prototrophy, and the resulting recombinants were analyzed for coinheritance of the *fla* mutation. Results of these experiments indicated that *flaA104* was cotransduced with *leuA* at a frequency of 11% and with *argG* at frequency of 5%. Since *leuA* and *argG* are cotransduced at a frequency of 19%, the most likely map order is *flaA—leuA—argG* as depicted in Figure 1. In previous experiments, we had shown the order *flaA—argG—Tn7* (ELY 1982). In addition, BARRETT *et al.* (1982a) had shown that *proA* was near *argG*, so phage grown on *argG* and *flaA* mutants were used to transduce *proA* mutants. The *proA* mutation was cotransduced with *flaA* at a frequency of 1% (12 of 761 transductants analyzed), but no cotransduction was observed between *proA* and *argG*. Therefore, we conclude that the order for the entire region is *proA—flaA—leuA—argG—Tn7*.

Phage grown on 15 additional strains containing *fla* mutations which had been classified as *flaA* by JOHNSON and ELY (1979) were used to transduce SC383 (*leuA*) to prototrophy. Linkage values obtained from these experiments could be classified into two groups. One group (*fla-103, fla-105, fla-107, fla-109, fla-118, fla-121, fla-136, fla-145, fla-147*) had an average transductional linkage of 11% to *leuA* as was found for *flaA104*. This group included all of the *flaA* stub mutants identified by JOHNSON and ELY (1979). The second group (*fla-111, fla-112, fla-114, fla-122, fla-139*) had an average transductional linkage of 37% to *leuA*). Since this value was significantly different from that obtained with the other *flaA* mutations, the *fla* mutations in these strains were designated *flbC*. Since representative *flbC* strains had linkage values similar to those obtained with SC229 (*flaA104*) in crosses with *proC* or *argG* mutants (data not shown), we conclude that *flbC* is between *flaA* and *leuA* (Figure 2).

Precise location of flaB, flaC, flaD, *and* motC: JOHNSON and ELY (1979) had demonstrated previously by transduction that mutations in *flaB, flaC* and *flaD* were linked to each other. We confirmed this result by demonstrating that

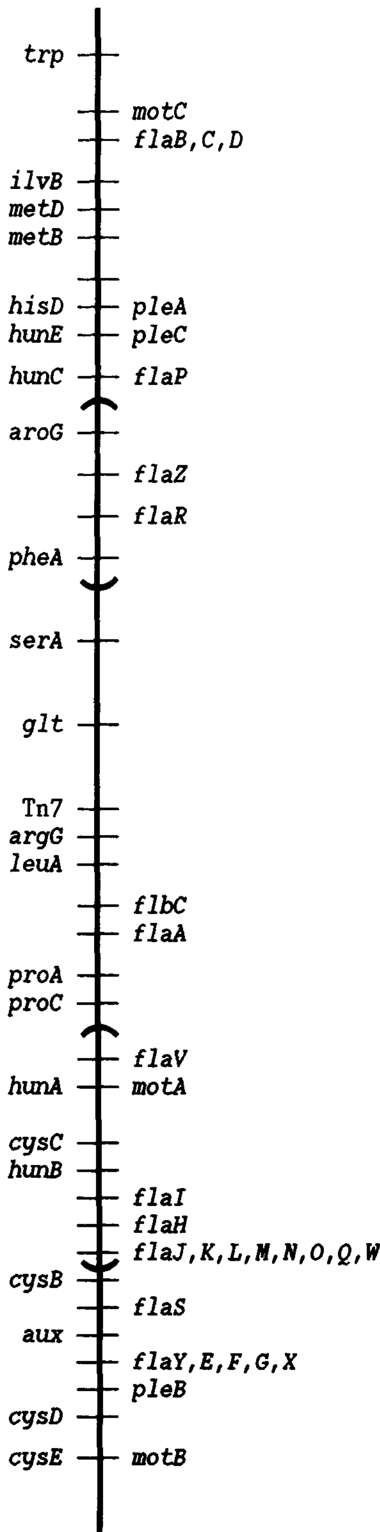

FIGURE 2.—Summary of the map positions of the *fla*, *mot* and *ple* mutations.

flaB160, *flaC108* and *flaD115* were linked to *ilvB*, resulting in cotransduction frequencies of 16, 11 and 18%, respectively. To determine on which side of *ilvB* the *fla* mutations were located, phage grown on strains containing each of the three *fla* mutations were used to transduce a *metD* auxotroph. Since linkage of 3–6% was observed between the *fla* mutations and *metD*, and since *metD* and *ilvB* are 26% linked (BARRETT *et al.* 1982a), we conclude that *ilvB* is between *metD* and the *flaBCD* gene cluster. Thus, the gene order in the region is *trpC—fla(BCD)—ilvB—metD* as shown in Figure 2. No transductional linkage was observed between *trpC* and any of the *fla* mutations, indicating that *trpC* is too far from *flaBCD* for transductional linkage. Experiments with the eight additional *flaC* and *flaD* mutants described by JOHNSON and ELY (1977) resulted in linkage values similar to those described for *flaC108* and *flaD115* (data not shown).

Experiments with the three *motC* mutants SC303, SC398 and SC509 showed that these mutations are located in the vicinity of the *flaBCD* genes. Transductions with phage grown on each of the three strains indicated linkages of 6% to *ilvB* and 1% to *metD*. Since the transductional linkages observed between mutations in the *flaBCD* gene cluster and *ilvB* were approximately 15%, we conclude that *motC* is farther from *ilvB* than the *flaBCD* genes are, and that the order for the region is *trpC—motC—fla(BCD)—ilvB—metD* as depicted in Figure 2.

Precise location of flaE, flaF, flaG, flaT, flaX, flaY *and* pleB: The *flaE*, *flaF*, and *flaG* genes were thought to be close to one another since deletion mutants were obtained that failed to give rise to motile recombinants in crosses with either *flaE* or *flaF* mutants and resulted in few motile recombinants with *flaG* mutants (JOHNSON and ELY 1979). Since *flaE* was shown to be near *cysD* in conjugation experiments, we used phage grown on a *cysD*::Tn*5* mutant to demonstrate transductional linkage to SC519 (*flaE163*), SC279 (*flaF132*) and SC278 (*flaG131*) (Table 2). Similar experiments with a strain containing a *cysE*::Tn*5* mutation failed to demonstrate transductional linkage between *cysE* and any of the three *fla* mutations. Since other mutations that are located between *cysD* and *cysE* all appear farther from the *flaE*, *flaF*, and *flaG* mutations than from *cysD* (data not shown), we conclude that the *flaE*, *flaF*, and *flaG* mutations are not located between *cysD* and *cysE*. To confirm that *flaE*, *flaF*, and *flaG* are on the *cysB* side of *cysD*, phage grown on each of the three *fla* mutants were used to demonstrate transductional linkage to an *aux* (unidentified nutritional requirement) mutation which is located between *cysD* and *cysB* (BARRETT *et al.* 1982a) (Table 2). Since conjugation experiments indicated that the *flaT*, *flaX* and *flaY* mutations are also near *cysD*, similar experiments were performed with strains containing these mutations. The linkage values obtained are similar to those obtained with the *flaE*, *flaF* and *flaG* mutations (Table 2), and we conclude that all six mutations are located in a cluster (Figure 2).

The *pleB* mutations also were localized in the vicinity of *cysD* from conjugation experiments. Consequently, phage grown on SC1091 (*cysD137*::Tn*5*) were used to transduce SC603 (*pleB132*) to kanamycin resistance and demonstrated 75% linkage between the two markers. Additional experiments dem-

TABLE 2

Transductional linkage values of fla *and* ple *mutations to* cysD, cysE *and* aux

Motility marker	% cotransduction to		
	cysD[a]	*cysE*[a]	*aux*[b]
flaE	29	0	7
flaF	23	0	3
flaG	21	0	2
flaT	9	0	9
flaX	35	0	5
flaY	16	0	5
pleB	75	ND[c]	ND[c]

[a] Phage grown on SC1091 (*cysD*::Tn*5*) or SC1489 (*cysE*::Tn*5*) marker were used to transduce a strain containing the motility marker to kanamycin resistance. Recombinants were screened for the presence of motile bacteria.

[b] Phage grown of the motility marker were used to transduce SC126 (*aux*) to prototrophy. Recombinants were screened for the presence of motile bacteria.

[c] ND, not determined.

onstrated that the other four *pleB* mutations were also closely linked to *cysD* (data not shown). Thus, *pleB* is in the same region as the *flaEFGTXY* gene cluster but appears to be located much closer to *cysD*.

Precise location of flaH, flaI, flaJ, flaK, flaL, flaM, flaN, flaO, flaQ, flaV, flaW *and* motA: Eleven *fla* genes and *motA* were linked to *cysB* but not to *cysE* by conjugation. To determine a more precise location for these genes, transduction experiments using phage grown on strains containing the *fla* mutations were used to transduce SC436 (*cysC*) to prototrophy. Unfortunately, the *cysC* mutation in this strain reverts at a high frequency and could not provide reliable data. Therefore, we sought other markers in the immediate vicinity of *cysC*. Mutations causing a reduced ability to use histidine as a nitrogen source (*hun*) had been isolated and were found to occur at a variety of sites scattered on the *C. crescentus* genetic map (D. FERBER and B. ELY, unpublished results). Two of these mutations, *hunA106* and *hunB109* had been shown to be linked to *cysC* by transduction (D. FERBER, unpublished data). Since the *hun* mutations were obtained by Tn*5* mutagenesis (ELY and CROFT 1982), phage grown on strains containing *hunA106*::Tn*5* or *hunB109*::Tn*5* could be used to transduce a strain of interest to kanamycin resistance on complex medium. When such an experiment was performed with SC436, we were able to confirm that *hunA106* and *hunB109* were transductionally linked to *cysC104* at frequencies of 12 and 1%, respectively. Transductions were then performed between phage grown on the two *hun* mutants and each of the *fla* and *mot* mutants in the region, and kanamycin resistant colonies were screened for motility (Table 3). Three of the mutations, *flaV140*, *motA101* and *motA102* were linked to the *hunA* mutation but not to the *hunB* mutation. The remaining strains were linked to the *hunB* mutation but not to the *hunA* mutation.

TABLE 3

Transductional linkage values of cysC, fla *and* mot *mutations to* hunA *and* hunB[a]

Recipient marker	% cotransduction to	
	hunA	*hunB*
cysC	12	1
flaH	0	56
flaI	0	70
flaJ	0	6
laK	0	11
flaL	0	11
flaM	0	15
flaN	0	12
flaO	0	11
flaQ	0	9
flaV	22	0
flaW	0	18
motA	64	0

[a] Phage grown on a strain containing the donor marker were used to transduce a strain containing the recipient marker to kanamycin resistance. Recombinants were screened for the presence of motile bacteria.

Of these, *flaI* seemed to be closest to *hunB* with a transductional linkage of 70%, followed by *flaH* with a linkage of 56%. Linkage of the other *fla* mutations to *hunB* ranged from 10 to 20%. Taken together the data indicate the gene arrangement shown in Figure 3.

Precise location of flaP, flaR, flaU, flaZ *and* pleC: Conjugation experiments with *flaP*, *flaR*, *flaU* and *flaZ* demonstrated that these markers are located in the vicinity of *pheA* and *aroG*. Previous experiments had shown that *flaZ* was located between *serA* and *hisD* (BARRETT *et al.* 1982b) and that *pheA* and *aroG* also were located between *serA* and *hisD* (BARRETT 1981). To determine more precise map positions for these genes, phage were grown on each of the *fla* mutants and used to transduce SC393 (*aroG109*) and SC1300 (*pheA108*) to prototrophy. The *flaZ* mutation was found to be linked to *aroG* and *pheA* at frequencies of 4 and 14%, respectively (Figure 4). In contrast, the *flaR* mutation was 25% linked to *pheA* but was unlinked to *aroG* by transduction. The order that best fits the linkage data is *aroG—flaZ—flaR—pheA* as shown in Figure 4.

Two *hun* mutations, *hunC115*::Tn*5* and *hunE112*::Tn*5*, were shown to be linked to *aroG* by conjugation at frequencies of 12 and 4%, respectively. Therefore, phage grown on strains containing these two *hun* mutations were used to transduce the *fla* and *pleC144* mutations to kanamycin resistance. No linkage was observed with either *flaR* or *flaZ*, but both *flaP* and *flaU* were found to be linked to *hunC* at a frequency of 27% and to *hunE* at a frequency of 4%. The *pleC144* mutation was 77% linked to *hunE* and was not linked to *hunC* (Figure 4). Eight additional *ple* mutations were transduced with phage

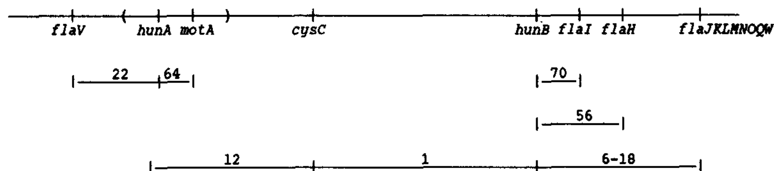

FIGURE 3.—Linkage relationships in the *cysC* region of the *C. crescentus* chromosome. Numbers represent percent cotransduction.

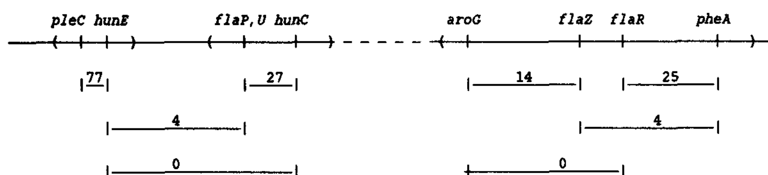

FIGURE 4.—Linkage relationships in the *pheA*—*aroG* region of the *C. crescentus* chromosome. Numbers represent percent cotransduction.

grown on the *hunE* mutant. All eight had approximately the same linkage to *hunE* as *pleC144*, confirming the *pleC* designation for these mutants.

Precise location of motB: Conjugation experiments demonstrated that the *motB* gene was located in the vicinity of *cysE*. Therefore, phage grown on SC286 (*motB103*) were used to transduce SC1489 (*cysE103*) and SC1091 (*cysD137*) to prototrophy. When the resulting transductants were screened for motility, *motB* was found to be 35% linked to *cysE* and 3% linked to *cysD*.

Precise location of pleA: Conjugation experiments indicated that the *pleA* gene was close to *hisD*. Consequently, phage grown on SC611 were used to transduce SC458 (*hisD117*) and SC459 (*metB115*) to prototrophy. When the resulting colonies were tested for motility, *pleA* was found to be 27% linked to *hisD* and 6% to *metB*. The transductional linkage value between these two markers is 2%. Therefore, we conclude that *pleA* is located between *hisD* and *metB* (Figure 2). When the other mutations designated *pleA* (Table 1) were crossed with SC458, similar linkage values were obtained confirming the *pleA* designation.

DISCUSSION

The flagellar genes have been studied in detail in only a few bacteria. In *Escherichia coli* and *Salmonella typhimurium* nearly all of the flagellar genes are found in three clusters in one part of the chromosome (SILVERMAN and SIMON 1977). In *Pseudomonas aeruginosa*, all of the known flagellar mutations are complemented by two R'*fla* plasmids (TSUDA, OGUCHI and IINO 1981). In contrast, the *C. crescentus fla* genes were scattered throughout the genome. There are three multigene clusters in three different parts of the genome, but, in addition, there are 11 *fla* or *mot* genes that are located at eight additional sites scattered around the chromosome (Figure 2). Thus, the flagellar genes of *C. crescentus* are much more scattered than has been observed in other bacteria.

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