GENERAL NONCHEMOTACTIC MUTANTS OF CAULOBACTER CRESCENTUS

BERT ELY,* CONNIE J. GERARDOT,* DONNA L. FLEMING,*
SUELY L. GOMES,†,1 PETER FREDERIKSE† AND LUCILLE SHAPIRO†

*Department of Biology, University of South Carolina, Columbia, South Carolina 29208, and
†Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of
Medicine, Bronx, New York 10461

ABSTRACT

We have examined 35 mutants that have defects in general chemotaxis. Genetic analysis of these mutants resulted in the identification of at least eight *che* genes located at six different positions on the *Caulobacter crescentus* chromosome. The *cheR*, *cheB* and *cheT* genes appeared to be located in a three-gene cluster. Mutations in these three genes resulted in the inability of the flagellum to reverse the direction of rotation. Defects in the *cheR* gene resulted in a loss of the ability to methylate the methyl-accepting chemotaxis proteins. *In vitro* experiments showed that the lack of *in vivo* methylation in *cheR* mutants was due to the absence of methyltransferase activity. Defects in the *cheB* gene resulted in greatly reduced chemotaxis-associated methylation *in vivo* and a loss of methylesterase activity *in vitro*. The specific defects responsible for the lack of a chemotactic response have not been determined for the other identified *che* genes.

MOTILITY has proven to be a convenient trait to monitor in studies of the *Caulobacter crescentus* cell cycle. The motility of *C. crescentus* cells is easily observed on semisolid agar plates or with a phase contrast microscope and is found to occur only in one stage of the cell cycle. Nonmotile mutants are readily isolated, and to date, over 30 genes affecting motility have been identified (ELY, CROFT and GERARDOT 1984). Furthermore, the *C. crescentus* flagellum is released from swarmer cells immediately before stalk formation and can be recovered intact from the culture medium. Studies of the flagellum have shown that the flagellin and hook proteins are synthesized and assembled midway through DNA replication in stalked cells (OSLEY, SHEFFERY and NEWTON 1977; AGABIAN, EVINGER and PARKER 1979; OSLEY and NEWTON 1980). Thus, the flagellin proteins are synthesized immediately before the time when the cell begins to swim.

An important aspect of bacterial motility is chemotaxis, the ability to respond to a chemical gradient. Mutants defective in chemotaxis (*che*) have normal

---

[1] Present address: Instituto de Quimica, Universidade de São Paulo, São Paulo, Brasil.

motility when viewed by phase microscopy, but fail to form swarms in semisolid medium. The mechanism by which a bacterium senses its environment is not fully understood, but adaptation to chemical stimuli in *E. coli* has been shown to be correlated with the reversible methylation of specific membrane proteins (SILVERMAN and SIMON 1977; SPRINGER, GOY and ADLER 1977). We have shown that *C. crescentus* carries out a similar methylation of membrane proteins and that the ability to methylate these membrane proteins is lost in certain mutants defective for chemotaxis (SHAW *et al.* 1983). Since mobility is cell-cycle-dependent in *C. crescentus*, we determined when during the cell cycle the chemotaxis-associated methylation occurred. We found that the methylated chemotaxis proteins (MCPs) and the enzymes involved in methylation and demethylation were synthesized during the period of flagellum biogenesis and were lost when the flagellum was shed (SHAW *et al.* 1983; GOMES and SHAPIRO 1984). Thus, at least a portion of the chemotaxis machinery is synthesized in a cell-cycle-dependent fashion.

In this report, we present a characterization of 35 spontaneous and Tn5-induced chemotaxis mutants representing at least eight genetic loci. The swimming behavior of representative mutants has been analyzed with respect to reversal frequency and the direction of flagellar rotation. Each mutant has also been tested for the ability to carry out *in vivo* methylation of the membrane MCPs. Two types of mutants that cannot carry out MCP methylation *in vivo* were shown to be unable to reverse swimming direction and to lack methyltransferase and methylesterase activity, respectively, *in vitro*. These adjacent genes have been designated *cheR* and *cheB*, in keeping with the comparable alleles in *Escherichia coli* and *Salmonella typhimurium* (SPRINGER and KOSHLAND 1977; STOCK and KOSHLAND 1978).

MATERIALS AND METHODS

The bacterial strains used in this study are described in Table 1. Nonswarming mutants were isolated as described by JOHNSON and ELY (1979) and ELY and CROFT (1982). Mutants defective in chemotaxis were identified as nonswarming mutants that had normal motility when observed by phase contrast microscopy, exhibited a normal cell cycle and had a doubling time comparable to wild type. The swarm size was the same, independent of phosphate concentration in the plates. 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 ØCr30 (ELY and JOHNSON 1977). Linkage values in the text represent the average of two or more independent determinations. Except in preliminary experiments, approximately 100–200 recombinants were analyzed for the presence of unselected markers in each determination.

In vivo methylation was carried out as described previously (SHAW *et al.* 1983) based on the procedure of KORT *et al.* (1975). Measurement of methyltransferase activity *in vivo* and *in vitro* was as described previously (GOMES and SHAPIRO 1984). Methylesterase was assayed as described by GOMES and SHAPIRO (1984).

Swimming behavior analysis of swarmer cells: Swarmer cells were separated from stalked and predivisional cells by centrifugation at 13,000 rpm in an Eppendorf centrifuge for 2–5 min. The isolated swarmer cells were monitored at 23° using dark field illumination. The cells were viewed using a video camera (RCA New Vicon lin.) mounted on the microscope. A computerized cell-tracking system (Motion Analysis Systems, Santa Rosa, California) was used to monitor swimming behavior.

TABLE 1

Bacterial strains

Strain	Genotype	Derivation or source
Caulobacter crescentus		
CB15	Wild type	POINDEXTER (1964)
SC116	*gltA101*	BARRETT *et al.* (1982b)
SC117	*ilvB101*	BARRETT *et al.* (1982b)
SC126	*aux*	BARRETT *et al.* (1982a)
SC141	*metD104*	BARRETT *et al.* (1982a)
SC374	*purB104*	BARRETT *et al.* (1982b)
SC451	*proC104*	Spontaneous in CB15
SC545	*lysA103*	BARRETT *et al.* (1982a)
SC714	*gltA101 rif-104*	Rif in SC116
SC1078	*trpB108::Tn5 str-152*	ELY and CROFT (1982)
SC1091	*cysD137::Tn5 str-152*	ELY and CROFT (1982)
SC1140	*lacA101::Tn5 str-152*	ELY and CROFT (1982)
SC1238	*ilvB126::Tn5 cysB102 str-142*	ELY and CROFT (1982)
SC1383	*ts-104* (pVS1)	BARRETT *et al.* (1982a)
SC1388	*aux rif-148*	BARRETT *et al.* (1982a)
SC1556	*lysA103 rif-192*	Rif in SC545
SC1581	*hunG105::Tn5 str-152*	D. M. FERBER and B. ELY (unpublished results)
SC1582	*hunA106::Tn5 str-152*	D. M. FERBER and B. ELY (unpublished results)
SC1585	*hunB109::Tn5 str-152*	D. M. FERBER and B. ELY (unpublished results)
SC1588	*hunE112::Tn5 str-152*	D. M. FERBER and B. ELY (unpublished results)
SC1591	*hunC115::Tn5 str-152*	D. M. FERBER and B. ELY (unpublished results)
Chemotaxis mutants		
SC1040	*cheB137::Tn5 str-152*	ELY and CROFT (1982)
SC1057	*cheB144::Tn5 proA103 str-140*	ELY and CROFT (1982)
SC1163	*cheB148::Tn5 str-152*	ELY and CROFT (1982)
SC291	*cheB118*	JOHNSON and ELY (1979)
SC1119	*cheJ152::Tn5 str-152*	ELY and CROFT (1982)
SC1124	*cheJ153::Tn5 str-152*	ELY and CROFT (1982)
SC152	*cheL126*	JOHNSON and ELY (1979)
SC304	*cheN119*	JOHNSON and ELY (1979)
SC273	*cheP113*(ts)	JOHNSON and ELY (1979)
SC276	*cheP115*	JOHNSON and ELY (1979)
SC521	*cheP121*	JOHNSON and ELY (1979)
SC232	*cheR101*	JOHNSON and ELY (1979)
SC245	*cheR107*	JOHNSON and ELY (1979)
SC250	*cheR108*	JOHNSON and ELY (1979)
SC251	*cheR109*	JOHNSON and ELY (1979)
SC254	*cheR110*	JOHNSON and ELY (1979)
SC267	*cheR112*	JOHNSON and ELY (1979)
SC275	*cheR114*	JOHNSON and ELY (1979)
SC1050	*cheR138::Tn5 proA103 str-140*	ELY and CROFT (1982)
SC1063	*cheR141::Tn5 proA103 str-140*	ELY and CROFT (1982)

TABLE 1—Continued

Strain	Genotype	Derivation or source
Chemotaxis mutants—continued		
SC1064	*cheR142::Tn5 proA103 str-140*	ELY and CROFT (1982)
SC1130	*cheR151::Tn5 cysB102 str-142*	ELY and CROFT (1982)
SC234	*cheS103*	JOHNSON and ELY (1979)
SC522	*cheS122*	JOHNSON and ELY (1979)
SC523	*cheS123*	JOHNSON and ELY (1979)
SC525	*cheS125*	JOHNSON and ELY (1979)
SC233	*cheT102*	JOHNSON and ELY (1979)
SC238	*cheT104*	JOHNSON and ELY (1979)
SC241	*cheT105*	JOHNSON and ELY (1979)
SC242	*cheT106*	JOHNSON and ELY (1979)
SC283	*cheT116*	JOHNSON and ELY (1979)
SC289	*cheT117*	JOHNSON and ELY (1979)
SC178	*che-129*	JOHNSON and ELY (1979)
SC263	*che-111*	JOHNSON and ELY (1979)
SC524	*che-124*	JOHNSON and ELY (1979)
Escherichia coli		
NC5403	C600 (pLSG261) (RP4)	P. V. SCHOENLEIN *et al.* (unpublished results)

The Motion Analysis System digitizes video images frame by frame and tracks the path of individual cells from one frame to the next. Data are collected on all cells in a given field of view for 5-sec intervals at a rate of 10 frames per second. Reversals were detected as an abrupt change in the rate of change of swimming direction between two frames. Detection of reversals closely correlated with reversals assessed by visual tracking of cells.

Determination of swimming direction of predivisional cells was made by direct observation of mid-log phase cultures of cells in bright field illumination at 1200 power magnification. Direction of swimming was detected by observing the position of the polar stalk in predivisional cells during swimming.

RESULTS

Isolation of *che* mutants: Previous studies in our laboratory resulted in the isolation of nonswarming mutants (JOHNSON and ELY 1979; ELY and CROFT 1982). These mutants were identified by the absence of swarming ability on complex semisolid medium. The formation of a swarm on an agar plate requires that the cell be able to respond to a chemotactic gradient in addition to having the ability to swim. Therefore, each of the presumptive nonmotile mutants was examined by phase contrast microscopy for the ability to swim. Mutants that failed to form a swarm on semisolid medium, but had normal motility and numbers of motile cells comparable to wild type when viewed in the phase contrast microscope, were considered to be chemotaxis mutants (for example, *cheR* in Figure 1). Since the initial screening for swarming was performed in rich medium, all of the mutants were presumed to have defects in the general chemotaxis machinery, rather than an altered response to a specific chemoattractant. This assumption was strengthened by showing that each of the mutants had an altered chemotactic response on semisolid minimal media
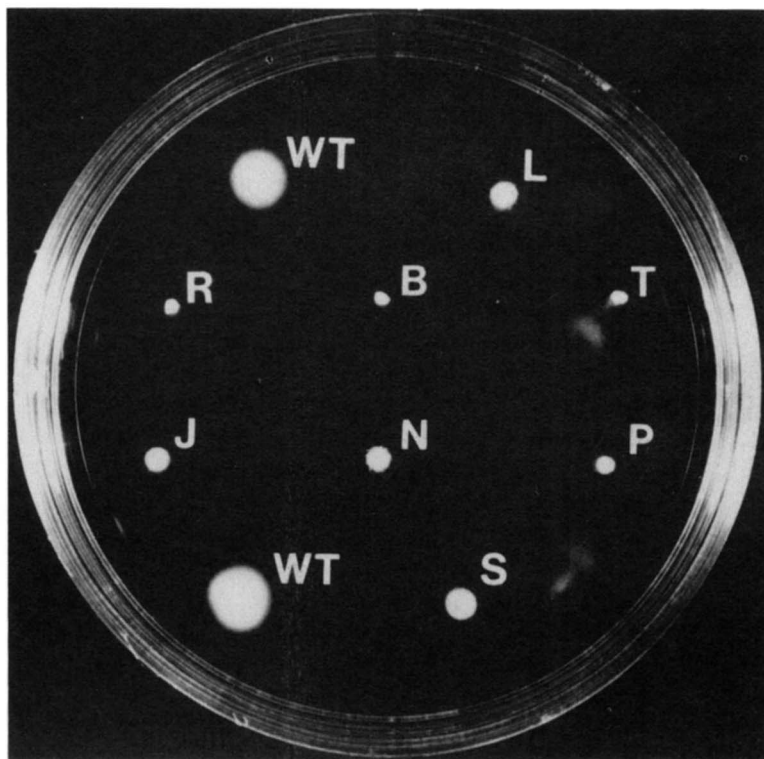
FIGURE 1.—Assay of general chemotactic behavior on semisolid agar swarm plates. Cultures of *C. crescentus* CB15 (wild type), *cheL* (SC152), *cheR* (SC1063), *cheB* (SC1040), *cheT* (SC238), *cheJ* (SC1124), *cheN* (SC304), *cheP* (SC226) and *cheS* (SC234), were stabbed onto 0.35% agar plates made with rich PYE medium.

containing glucose, xylose or alanine as the attactant. Some of the specific chemoattractants identified for *C. crescentus* include glucose, galactose, xylose, ribose, alanine, proline and glutamine.

Swimming behavior of wild-type and mutant cells: In contrast to *E. coli*, which has peritrichous flagella, *C. crescentus* has a single polar flagellum. In *E. coli*, the direction of flagella rotation elicits a specific swimming pattern. Rotation in the counterclockwise direction results in the formation of a flagellar bundle and smooth swimming. A switch to clockwise rotation results in loss of coordination of the flagellar bundle and the bacteria tumble. In *C. crescentus*, a switch in the direction of rotation of the polar flagellum causes the bacteria to swim in the reverse direction. Thus, *C. crescentus* can produce translational movement in either the forward or the reverse direction. When wild-type *C. crescentus* are observed by phase contrast microscopy, predivisional and swarmer cells can be seen to undergo rapid reversals of direction. The long forward swim appears to be comparable to the smooth swim exhibited by peritrichously flagellated bacteria, and the short backward swim appears to be comparable to the tumbling behavior. During the long forward swim, the predivisional cell swims with the stalked end in front. The reversal frequency

TABLE 2

Swimming behavior of wild-type and chemotaxis mutants

Strain	Genotype	Reversal frequency (reversals per cell/5 sec)	Flagellar rotation
CB15	Wild type	0.50	CW/CCW[a]
SC1130	*cheR*	0	CW
SC1064	*cheR*	0	CW
SC1063	*cheR*	0	CW
SC1163	*cheB*	0	CCW
SC1040	*cheB*	0	CCW
SC1057	*cheB*	0	CCW
SC238	*cheT*	0	CCW
SC1124	*cheJ*	0.54	CW/CCW
SC234	*cheS*	0.70	CW/CCW
SC304	*cheN*	0.55	CW/CCW
SC152	*cheL*	0.62	CW/CCW

[a] CW, Clockwise rotation; CCW, counterclockwise rotation. CW rotation yields a long forward swim, and CCW rotation yields a short reverse swim.

of wild-type *C. crescentus* and mutants representative of each *che* locus were analyzed in a computerized cell-tracking system, as described in MATERIALS AND METHODS (Table 2). Of the seven mutants tested, only three, *cheR, cheB* and *cheT*, were unable to reverse swimming direction. The other mutants had reversal frequencies comparable to wild type. Of the mutants unable to reverse direction, the *cheR⁻* strains swam only in the forward (stalked end in front) direction, whereas the *cheB⁻* strains appeared to be locked in the reverse (flagellar end in front) direction, KOYASU and SHIRAKIHARA (1984) showed that the forward swim in *C. crescentus* is due to clockwise flagellar rotation. Based on this information, the direction of flagellar rotation is indicated for each mutant listed in Table 2.

In vivo **methylation of the *C. crescentus* MCPs:** It was shown previously that several membrane MCPs in wild-type *C. crescentus* formed alkali-labile carboxylmethylesters of glutamate residues both *in vivo* and *in vitro* (GOMES and SHAPIRO 1984). Each of the chemotaxis mutants was tested for the ability to incorporate [*methyl*-³H]methionine into the MCPs in the absence of protein synthesis. Representative data are shown in Figure 2. Mutants designated *cheR* did not incorporate alkali-labile ³H-methyl groups into membrane proteins. As shown below, *cheR* mutants were found to lack methyltransferase activity. Mutations in the *cheB* gene resulted in low levels of MCP methylation, and the methylation that did occur was predominately in only one of the MCP bands. Because the *cheB* gene appears to encode the methylesterase (see below), the reduced *in vivo* incorporation of ³H-methyl groups probably reflects the fact that the sites normally available for methylation are filled in these mutant strains. Mutations in most of the remaining *che* genes resulted in normal patterns of methylation of the MCPs, although methylation was not observed in SC152 (*cheL*) (Figure 2).
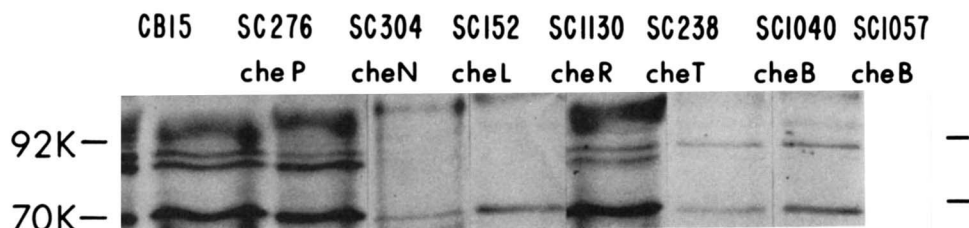
CB15 SC276 SC304 SC152 SC1130 SC238 SC1040 SC1057

cheP cheN cheL cheR cheT cheB cheB

92K—

70K—

FIGURE 2.—*In vivo* incorporation of alkali-labile ^{3}H-methyl into membrane methyl-accepting chemotaxis proteins of *C. crescentus* CB15 (wild type) and seven mutants derived from this strain by Tn5 mutagenesis: *cheP* (SC276), *cheN* (SC304), *cheL* (SC152), *cheR* (SC1130), *cheT* (SC238), *cheB* (SC1040) and *cheB* (SC1057). *In vivo* methylation was carried out as described previously (SHAW *et al.* 1983) based on the procedure of KORT *et al.* (1975). Cultures were incubated in the presence of [*methyl*-^{3}H]methionine (75 μCi/ml) under conditions in which protein synthesis was inhibited by chloramphenicol (50 μg/ml). Shown are autoradiographs of SDS polyacrylamide gel electrophoretograms of the labeled cells.

TABLE 3

Activity of proteins involved in chemotaxis-mediated methylation

Strain	Methyltransferase[a] (pmol/mg/ 30′)	Methylesterase[b] (% wild type)	MCP[a] (pmol/mg/ 30′)
CB15 (wild type)	56	100	56
SC1130 (*cheR*)	3	25	10
SC1040 (*cheB*)	36	16	47

[a] Methyltransferase activity and methyl-accepting chemotaxis protein (MCP) activity was measured *in vitro* according to SPRINGER and KOSHLAND (1977) and was modified as described by GOMES and SHAPIRO (1984). The incorporation of 1 pmol of methyl-^{3}H from S-adenosyl-L-[^{3}H]methionine (4×10^5 cpm/pmol/μg) of soluble fraction (methyltransferase) is, in each case, shown per milligram of the membrane fraction [methyl-accepting chemotaxis protein (MCP)].

[b] Methylesterase activity was measured as described by GOMES and SHAPIRO (1984). Membranes methylated *in vivo* were used as substrate for soluble fractions prepared from the wild-type and mutant cell extracts. After stopping the reaction, the membrane proteins were separated by SDS gel electrophoresis, and autoradiograms of the gels were submitted to densitometric scanning to monitor the loss of ^{3}H label from the MCPs.

In vitro methylation of the *C. crescentus* MCPs: The methyltransferase and methylesterase activities have been measured *in vitro* in wild-type strains of *C. crescentus*, and both activities were found to be present in swarmer cells, but not in stalked cells (GOMES and SHAPIRO 1984). *C. crescentus* strains carrying mutations in *cheR* and *cheB* were assayed for methyltransferase and methylesterase activities. The 11 *cheR* mutants listed in Table 1 were found to have little or no methyltransferase activity (Table 3; data not shown). Since all other mutants tested have wild-type levels of methyltransferase activity, the *cheR* gene is probably the structural gene for the methyltransferase. Mutants in *cheB* had

significantly reduced levels of methylesterase activity. It was shown previously that antibody raised against Salmonella methylesterase (J. B. STOCK, unpublished results) cross-reacts with the *C. crescentus* methylesterase (GOMES and SHAPIRO 1984). A specific protein of 38K was immunoprecipitated from wild-type *C. crescentus*, and this protein was missing from the mutant strain SC1040 (*cheB*::Tn5). It appears likely, therefore, that *cheB* is the structural gene for the methylesterase. A mutant carrying a Tn5 insertion in the *cheR* gene (SC1130) was also found to have reduced levels of methylesterase activity. Since these two mutations are located in the same region of the genetic map (see below), and within a 3-kb piece of cloned DNA (W. A. ALEXANDER and L. SHAPIRO, unpublished results), it is likely that this Tn5 mutation in the *cheR* gene has a polar effect on the expression of the *cheB* gene.

Based on the swimming mode of methyltransferase mutants (smooth), and methylesterase mutants (tumbly) in *E. coli*, one can predict that the *C. crescentus* methyltransferase mutants would be locked in forward swimming and methylesterase mutants would be locked in the reverse swimming mode; this indeed appears to be the case (Table 2).

General strategy for mapping *che* mutations: Since the *E. coli che* genes are clustered in the vicinity of the *fla* genes (SILVERMAN and SIMON 1977) and since some clustering of *C. crescentus fla* genes has been observed (ELY, CROFT and GERARDOT 1984), we tested the *che* genes for transductional linkage to markers in the vicinity of the flagellar genes. These experiments resulted in the identification of transductional linkage with mutations in seven genes, *cheJ*, *cheL*, *cheN*, *cheP*, *cheR*, *cheB* and *cheT*. In the case where no linkage was observed, additional experiments were performed to test for transductional or conjugational linkage in other regions of the chromosome. The approximate map position of each of the *che* genes is shown in Figure 3. The map positions of the *che* mutations in SC178, SC263 and SC524 have not been determined.

Precise location of the *cheR*, *cheB* and *cheT* genes: Preliminary transduction experiments indicated that mutations in *cheR*, *cheB* and *cheT* were linked to *proC*. Consequently, more detailed experiments were performed with each of the 20 *cheR*, *cheB* and *cheT* mutants listed in Table 1. The *cheR* and *cheB* mutants had transductional linkages of 4–17% to *proC104*, and the *cheT* mutants had transductional linkages of 15–25% to *proC104* (data not shown). In order to determine the map location more precisely, we used phage grown on several *cheR::Tn5* and *cheB::Tn5* mutants to transduce SC1383 (*ts-104*) to kanamycin resistance and obtained linkage values of 8–9% (Table 4). Similarly, when crosses with phage grown on *cheT* mutants were used to transduce SC1383 to temperature-insensitivity, linkage values of 8–12% were obtained (Table 4). Since *ts-104* and *proC* are not linked by transduction (BARRETT *et al.* 1982a), these results indicate that *cheR*, *cheB* and *cheT* are located between *ts104* and *proC*, but the relative order has not been determined.

Precise location of the *cheN* gene: Transduction experiments indicated that *cheN* was located in the vicinity of *hunA* with a contransductional linkage of 59% (Table 4). Since *motA* is 64% linked to *hunA* (ELY, CROFT and GERARDOT

aroF
trpE
flaD
flaB
flaC
motC
cheJ
ilvB
metB
hunE
hunC
cheL
flaP
aroG
flaZ
flaR
pheA
aroC
purB
hunG
cheS
trpB
gltA
aroB
purC
leuA
flbC
flbA
proA
ts104
cheT
cheB
cheR
proC
flaV
hunA
motA
cysC
cheN
hunB
flaW
flbF
flbD
flaO
flaK
flaI
flaH
flaG
cysB
flaJ
flaS
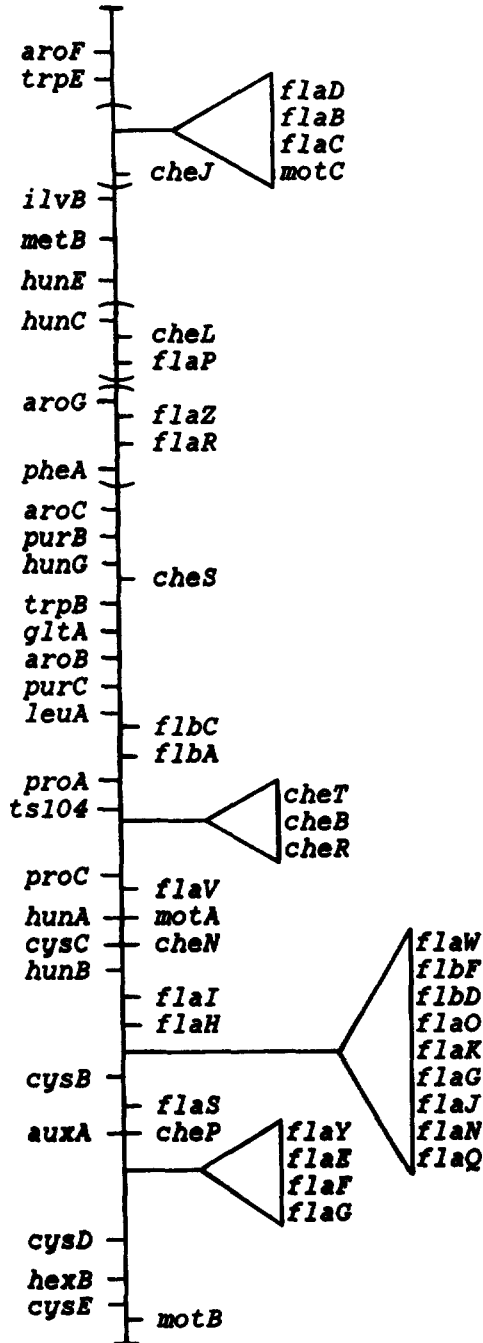flaN
auxA
cheP
flaY
flaE
flaF
flaG
flaQ
cysD
hexB
cysE
motB

FIGURE 3.—The map positions of the *che* genes reported in this paper are shown on the right relative to the previously determined positions of the *fla* and *mot* genes (ELY, CROFT and GERARDOT, 1984). The *che* genes are boxed. The map positions of marker genes are shown on the left (BARRETT *et al.* 1982a,b).

TABLE 4

Transductional crosses used to determine the map positions of *che* mutants

Donor	Recipient	% Cotransduction
SC1057 (*cheB144::Tn5*)	SC1383 (*ts-104*)	9
SC1040 (*cheB137::Tn5*)	SC1383 (*ts-104*)	9
SC1130 (*cheR151::Tn5*)	SC1383 (*ts-104*)	8
SC233 (*cheT102*)	SC1383 (*ts-104*)	8
SC238 (*cheT104*)	SC1383 (*ts-104*)	12
SC1582 (*hunA::Tn5*)	SC304 (*cheN119*)	59
SC273 (*cheP113*)	SC1091 (*cysD::Tn5*)	5
SC276 (*cheP115*)	SC1091 (*cysD::Tn5*)	5
SC521 (*cheP121*)	SC1091 (*cysD::Tn5*)	5
SC273 (*cheP113*)	SC1140 (*lacA*)	0
SC276 (*cheP115*)	SC1140 (*lacA*)	0
SC521 (*cheP121*)	SC1140 (*lacA*)	0
SC273 (*cheP113*)	SC1388 (*aux*)	6
SC276 (*cheP115*)	SC1388 (*aux*)	10
SC521 (*cheP121*)	SC1388 (*aux*)	9
SC1591 (*hunC115::Tn5*)	AE6002 (*pigA501*)	5
SC1588 (*hunE112::Tn5*)	SC152 (*cheL126*)	10
SC1591 (*hunC115::Tn5*)	SC152 (*cheL126*)	23
SC1119 (*cheJ152::Tn5*)	SC117 (*ilvB*)	18
SC1124 (*cheJ153::Tn5*)	SC117 (*ilvB*)	18
SC1119 (*cheJ152::Tn5*)	SC141 (*metD*)	14
SC1124 (*cheJ153::Tn5*)	SC141 (*metD*)	9
SC1581 (*hunG::Tn5*)	SC234 (*cheS103*)	23
SC1581 (*hunG::Tn5*)	SC522 (*cheS122*)	12
SC1581 (*hunG::Tn5*)	SC523 (*cheS123*)	20
SC1581 (*hunG::Tn5*)	SC525 (*cheS125*)	28
SC1078 (*trpB::Tn5*)	SC522 (*cheS122*)	15
SC1078 (*trpB::Tn5*)	SC523 (*cheS123*)	22
SC1581 (*hunG::Tn5*)	SC374 (*purB*)	8
SC1581 (*hunG::Tn5*)	SC1078 (*trpB*)	0

1984), it is possible that *motA* and *cheN* are located quite close together, either as adjacent genes or as alleles of the same gene.

Precise location of the *cheP* gene: Transduction experiments indicated that *cheP* was linked to *cysD* (Table 4). Further experiments demonstrated no linkage of the *cheP* mutations to *lacA* and 6–10% linkage to the *aux* marker (Table 4). Since the *flaYEFG* gene cluster is 20% linked to *cysD* and 5% linked to *aux* (ELY, CROFT and GERARDOT 1984), the *cheP* gene must be in the vicinity of this cluster, but closer to *aux*.

Precise location of the *cheL* gene: Preliminary experiments with SC152 (*cheL*) indicated conjugation linkage to the *lysA* gene. This result was confirmed by transduction experiments, which resulted in linkage values of 23% between *hunC* and *cheL* and 10% between *hunE* and *cheL*. Since *flaP* is located in a similar map position to *cheL* (ELY, CROFT and GERARDOT 1984), we tested SC152 for complementation by a cloned 5.6-kb fragment of *C. crescentus* DNA containing the *flaP* gene. NC5403 containing (pLSG261) was mated with
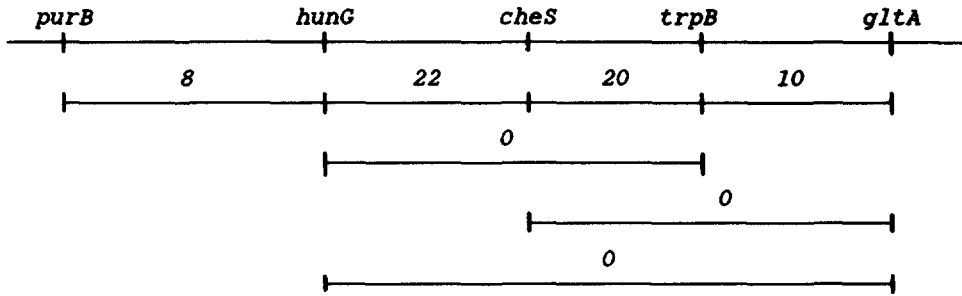
purB		hunG	cheS	trpB	gltA

```
         8          22         20        10
  +------+------------+---------+---------+
              0
         +-------------------------+
                        0
              +------------------------------+
                        0
              +---------------------------------+
```

FIGURE 4.—Map positions of the *cheS* gene determined by φCr30-mediated transduction. Numbers indicate percentage of cotransduction.

SC152, and the resulting transconjugants had regained the ability to swarm in semisolid medium. Thus, the *cheL* gene is located on the same DNA fragment as *flaP*.

Precise location of the *cheJ* gene: Transduction experiments indicated that *cheJ* was 18% linked to *ilvB* (Table 4). In order to determine the precise location of *cheJ*, transduction experiments were performed using SC141 (*metD104*) as a recipient, and linkage values of 14% with *cheJ153* and 9% with *cheJ154* were obtained (Table 4). Since *metD* and *ilvB* are approximately 15% linked by transduction (BARRETT *et al.* 1982a), these results suggest the map order *cheJ-ilvB-metD* and indicate that *cheJ* is in the vicinity of the *flaDBC-motC* gene cluster. However, complementation experiments with clones containing 24 kb of *C. crescentus* DNA failed to complement *cheJ* mutants, although they do complement *flaB*, *flaC*, *flaD* and *motB* mutants (K. HAHNENBERGER, personal communication).

Precise location of the *cheS* gene: Preliminary transduction experiments indicated that *cheS* was located in the vicinity of *hunG*. Therefore, SC1581 (*hunG105::Tn5*) and SC1078 (*trpB108::Tn5*) were crossed against the *cheS* mutants, and linkage values of 12–28% to *hunG* and 15–22% to *trpB* were obtained (Table 4). In contrast, no linkage was obtained between *cheS* and *gltA* (data not shown). Additional crosses demonstrated that *hunG* was 8% linked to *purB*, but no linkage was detected between *trpB105* and *hunG105* (Table 4). Previously we had demonstrated that *trpB* and *gltA* had a transductional linkage of 10% (WINKLER *et al.* 1984); thus, the order of markers in the region is *purB-hunG-cheS-trpB-gltA*, as shown in Figure 4.

DISCUSSION

In *E. coli*, seven genes involved in chemotaxis are located in a cluster at minute 42 on the *E. coli* genetic map (SILVERMAN and SIMON 1977). Based on the genetic map position and assays of methyltransferase and methylesterase activities, we have identified at least eight *C. crescentus* genes involved in chemotaxis in all attractants tested. Several genes for chemosensory functions, including those encoding the methyltransferase, the methylesterase and one of the MCPs, are expressed during a specific time segment of the cell cycle (SHAW *et al.* 1983; GOMES and SHAPIRO 1984), and their gene products are seques-

tered to a specific portion of the cell (GOMES and SHAPIRO 1984). It may be
that some *che* genes are involved in the temporal and spatial regulation of the
chemotaxis proteins, or that mutations in genes that are primarily involved
with other aspects of the cell cycle affect chemotaxis as well. Experiments with
additional mutants able to respond to some chemoattractants, but not to oth-
ers, have just begun.

A major phenotypic difference between the general *che* mutants in *E. coli*
(STOCK and KOSHLAND 1984) as compared to *C. crescentus* is that only three
of the eight *che* mutants in *C. crescentus* exhibit altered reversal frequencies
(Table 2), whereas the *E. coli* general *che* mutants all show changes in reversal
frequency. This difference may reflect the possibility that the mechanisms
controlling rotor reversal used by bacteria with a single flagellum are not
completely parallel to those used by peritrichously flagellated bacteria.

A second difference between *E. coli* and *C. crescentus* is that there is very
little clustering of the *che* genes in *C. crescentus*. Genetic experiments demon-
strated that the eight *che* genes were at six different locations scattered around
the chromosome (Figure 3). The one gene cluster that was detected involved
20 independent mutations located in at least three *che* genes, *cheR*, *cheB* and
cheT. Thus, it is possible that an additional *che* gene(s) may be located in this
region. Based on the swimming behavior of the *cheR* and *cheB* mutants, and
on *in vivo* and *in vitro* assays of MCP methylation and demethylation, the *cheR*
and *cheB* genes appear to encode the methyltransferase and methylesterase
involved in modulation of chemosensory transduction. These genes are adja-
cent to each other on the *C. crescentus* chromosome, as is the case in *E. coli*.
A third gene in the *C. crescentus* cluster, *cheT*, also appears to be involved in
the modulation of flagellar rotation, because *cheT* mutants are unable to re-
verse direction of swimming. Hybridization of the Salmonella *cheB* and *cheY*
genes to the cloned comparable region from *C. crescentus* suggests that *cheT*
might be analogous to the Salmonella *cheY* gene (W. A. ALEXANDER and L.
SHAPIRO, unpublished results). Transposon Tn*5* insertions in these genes show
polarity of gene expression and suggest that they are organized in an operon
in the same order as found in *E. coli* (W. A. ALEXANDER and S. L. GOMES,
unpublished results). The *cheRBT* gene cluster and most of the other *che* genes
are not located in the immediate vicinity of any flagellar genes. However, we
did find that four of the genes, *cheJ*, *cheL*, *cheN* and *cheP*, were located in
regions containing *fla* or *mot* genes.

Recently, it has been demonstrated that *Bacillus subilis* has at least 21 genes
involved in the general chemotactic response and that these genes are found
in a cluster separate from the flagellar genes (ORDAL, PARKER and KIRBY
1985). The role of these additional genes remains to be elucidated; however,
it has been shown that *B. subtilis* MCPs are demethylated, rather than meth-
ylated, in response to an attractant (ORDAL, PARKER and KIRBY 1985). From
this comparison it would appear that, although the basic features of chemotaxis
seem to have been conserved, the detailed features of the systems will vary
among the genera.

LITERATURE CITED

AGABIAN, N., M. EVINGER and E. PARKER, 1979 Generation of asymmetry during development. J. Cell. Biol. **81:** 123–126.

BARRETT, J. T., R. H. CROFT, D. M. FERBER, C. J. GERARDOT, P. V. SCHOENLEIN and B. ELY, 1982a Genetic mapping with Tn*5*-derived auxotrophs of *Caulobacter crescentus.* J. Bacteriol. **151:** 888–898.

BARRETT, J. T., C. S. RHODES, D. M. FERBER, B. JENKINS, S. A. KUHL and B. ELY, 1982b Construction of a genetic map for *Caulobacter crescentus.* J. Bacteriol. **149:** 889–896.

ELY, B., 1979 Transfer of drug resistance factors to the dimorphic bacterium *Caulobacter crescentus.* Genetics **91:** 371–380.

ELY, B. and R. H. CROFT, 1982 Transposon mutagenesis in *C. crescentus.* J. Bacteriol. **149:** 620–625.

ELY B., R. H. CROFT and C. J. GERARDOT, 1984 Genetic mapping of genes required for motility in *Caulobacter crescentus. Genetics* **108:** 523–532.

ELY, B. and R. C. JOHNSON, 1977 Generalized transduction in *Caulobacter crescentus.* Genetics **87:** 391–399.

GOMES, S. L. and L. SHAPIRO, 1984 Differential expression and positioning of chemotaxis methylation proteins in *Caulobacter.* J. Mol. Biol. **177:** 551–568.

JOHNSON, R. C. and B. ELY, 1979 Isolation of spontaneously derived mutants of *Caulobacter crescentus.* Genetics **86:** 25–32.

JOHNSON, R. C. and B. ELY, 1979 Analysis of nonmotile mutants of the dimorphic bacterium *Caulobacter crescentus.* J. Bacteriol. **137:** 627–634.

KORT, E. N., M. F. GOY, S. H. LARSEN and J. ADLER, 1975 Methylation of a membrane protein involved in bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **72:** 3939–3943.

KOYASU, S. and Y. SHIRAKIHARA, 1984 *Caulobacter crescentus* flagellar filament has a right-handed helical form. J. Mol. Biol. **173:** 125–130.

ORDAL, G. W., H. M. PARKER and J. R. KIRBY, 1985 Complementation and characterization of chemotaxis mutants of *Bacillus subtilis.* J. Bacteriol. **164:** 802–810.

OSLEY, M. A. and A. NEWTON, 1980 Temporal control of the cell cycle in *Caulobacter crescentus*: roles of DNA chain elongation and completion. J. Mol. Biol. **138:** 109–128.

OSLEY, M. A., M. SHEFFERY and A. NEWTON, 1977 Regulation of flagellin synthesis in the cell cycle to *Caulobacter*: dependence on DNA replication. Cell **12:** 393–400.

POINDEXTER, J. S., 1964 Biological properties and classification of the *Caulobacter* group. Bacteriol. Rev. **28:** 231–295.

SHAW, P., S. L. GOMES, K. SWEENEY, B. ELY and L. SHAPIRO, 1983 Methylation involved in chemotaxis is regulated during *Caulobacter* differentiation. Proc. Natl. Acad. Sci. USA **80:** 5261–5265.

SILVERMAN, M. and M. SIMON, 1977 Chemotaxis in *E. coli*: methylation of *che* gene products. Proc. Natl. Acad. Sci. USA **74:** 3317–3721.

SPRINGER, M. S., M. F. GOY and J. ADLER, 1977 Sensory transduction in *E. coli*: two comple-

mentary pathways of information processing that involve methylated proteins. Proc. Natl. Acad. Sci. USA **74:** 3312–3316.

SPRINGER, W. R. and D. E. KOSHLAND, JR., 1977 Identification of a protein methylesterase as the *cheR* gene products in the bacterial sensing system. Proc. Natl. Acad. Sci. USA **74:** 533–537.

STOCK, J. B. and D. E. KOSHLAND, JR., 1978 A protein methylesterase involved in bacterial sensing. Proc. Natl. Acad. Sci. USA **75:** 3659–3663.

STOCK, J. B. and D. E. KOSHLAND, JR., 1984 Sensory adaptation mechanisms in swarm development. Pp. 117–131. In: Microbial Development Edited by R. LOSICK and L. SHAPIRO. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

WINKLER, M. E., P. V. SCHOENLEIN, C. M. ROSS, J. T. BARRETT and B. ELY, 1984 Genetic and physical analyses of *Caulobacter crescentus trp* genes. J. Bacteriol. **160:** 279–287.