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Analysis of the pleiotropic regulation of flagellar and chemotaxis gene expression in *Caulobacter crescentus* by using plasmid complementation

(Tn*5* insertion mutations/chemotaxis methylation/flagellins)

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ABSTRACT **The biosynthesis of the single polar flagellum and the proteins that comprise the chemotaxis methylation machinery are both temporally and spacially regulated during the *Caulobacter crescentus* cell-division cycle. The genes involved in these processes are widely separated on the chromosome. The region of the chromosome defined by *flaE* mutations contains at least one flagellin structural gene and appears to regulate flagellin synthesis and flagellar assembly. The protein product of the adjacent *flaY* gene was found to be required to regulate the expression of several flagellin proteins and the assembly of a functional flagellum. We demonstrate here that each of these genes is also required for the expression of chemotaxis methylation genes known to map elsewhere on the chromosome. In order to study the regulation of these genes, plasmids were constructed that contain either an intact *flaYE* region or deletions in the region of *flaY*. These plasmids were mated into a wild-type strain and into strains containing various Tn*5* insertion and deletion mutations and a temperature-sensitive mutation in the *flaYE* region. The presence of a plasmid containing the *flaYE* region allowed the mutant strains to swim and to exhibit chemotaxis, to synthesize increased amounts of the flagellins, to methylate their "methyl-accepting chemotaxis proteins" (MCPs), and to regain wild-type levels of methyltransferase activity. Chromosomal deletions that extend beyond the cloned region were not complemented by this plasmid. Plasmids containing small deletions in the *flaY* region failed to restore to any *flaY* or *flaE* mutants the ability to swim or to assemble a flagellar filament. When mated into a wild-type strain, plasmids bearing deletions in the *flaY* region were found to be recessive. The pleiotropic regulation of flagellin synthesis, assembly, and chemotaxis methylation functions exhibited by both the *flaY* and *flaE* genes suggest that their gene products function in a regulatory hierarchy that controls both flagellar and chemotaxis gene expression.**

Bacterial flagella are composed of approximately 15 structural proteins and require on the order of 30 genes for their biogenesis and function (1, 2). A group of genes are also involved in chemotaxis functions. The genes devoted to the biogenesis of flagella likely mediate the complex regulation of their assembly, the coordination of their biogenesis with the cell-division cycle, and their positioning on the cell surface. The expression of groups of flagellar and chemotaxis genes has been shown in *Escherichia coli* to be controlled, in part, by a regulatory cascade (3). It has been demonstrated that the transcription of one group of *fla* genes is required for the expression of another group, and so on, in an hierarchical manner. The order of a *fla* gene in the cascade appears to correspond to the position of its protein product in the flagellar morphogenetic pathway (3, 4). Analysis in *E. coli* of second-site suppressors of specific *che* mutants has provided genetic evidence that certain *che* gene products directly interact with at least two components of the flagellar machinery (5). This kind of direct interaction may be part of the mechanism by which *che* gene expression is governed within the flagellar gene regulatory cascade (3).

The biogenesis of the *Caulobacter crescentus* single polar flagellum requires at least 26 genes (refs. 6 and 7; B. Ely, personal communication). The *fla*, *mot*, and *che* genes are located both singly and in several clusters widely scattered on the chromosome (see Fig. 1*A*). The flagellum has been shown to be synthesized and assembled at a given time in the cell-division cycle and to be released from the cell surface at a specific time later in the cell cycle (8–11). Some of us recently have demonstrated that the biochemical machinery that mediates chemotaxis exists only when the cell is able to respond to a chemotactic signal (7). This coincident turn-on of the *fla* and the *che* genes in *C. crescentus* may be analogous to the highly integrated control of these genes seen in *E. coli* and *Salmonella*. However, the *loss* of the *che* functions in *C. crescentus* coincident with the physical release of the flagellum from the cell surface indicates additional levels of control probably involving structural constraints.

C. crescentus contains several genes for flagellin proteins (12–14). The flagellar filament is composed of two major flagellins [25 and 27.5 kilodaltons (kDa)], and a 29-kDa flagellin appears to be a minor component required for filament assembly (12, 14, 15). The flagellin genes appear to be located at two distinct regions on the chromosome (14). The *flaYE* region of the chromosome includes at least one gene that encodes a flagellin protein (14, 16, 17). The sequence of the *flaE* gene has been determined recently and shown to encode the 29-kDa flagellin (ref. 14; see Fig. 1*C*). Other flagellin genes do not occur in the region directly 3′ to this gene (14). Several Tn*5* insertions 3′ to this structural gene and deletions that are missing sequences in the *flaYE* region (see Fig. 1 *B* and *C*) have been shown to result in a general decrease in the synthesis of all flagellins and to yield a Fla$^-$ phenotype (16, 18). A point mutation in *flaE* was found to result in the loss of the 29-kDa flagellin and simultaneously to lower the levels of the other flagellins. We show here that the mutant strains containing these insertions and deletions also curtail methylation functions associated with chemotaxis. The genes involved in chemotaxis methylation have been identified and mapped to other regions of the chromosome (7). Mutations in *flaY* and in *flaYE* resulted in a decrease in both methyltransferase activity and methyl-acceptor activity of the "methyl-accepting chemotaxis proteins" (MCPs).

As a first step in the analysis of these regulatory interactions, we report here plasmid complementation of this defined set of deletion and insertion mutants in the *flaYE* gene

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Abbreviations: MCPs, methyl-accepting chemotaxis proteins; kDa, kilodalton(s).

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cluster (see Fig. 1*A*). A temperature-sensitive mutation in *flaY* was also complemented. Mutant strains altered in the *flaYE* region but carrying a plasmid with an intact *flaYE* gene cluster were found to swim and undergo chemotaxis, to make increased amounts of flagellins, to methylate their MCPs, and to have wild-type levels of methyltransferase activity. The *flaY* gene product, which is required for normal levels of flagellin synthesis and flagellar assembly, also is required for chemotaxis methylation. These regulatory events are effected either directly (in *trans*) or indirectly by a cascade mechanism to control distal *fla* and *che* gene expression, demonstrating the coordinate control of these genes in *C. crescentus*.

MATERIALS AND METHODS

Materials. Calf alkaline phosphatase was obtained from Boehringer Mannheim. DNA polymerase I, T4 DNA ligase, and all restriction enzymes were obtained from New England BioLabs. 5′[α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq) and *S*-adenosyl-[*methyl*-^{3}H]methionine (15 Ci/mmol) were purchased from Amersham. [*methyl*-^{3}H]methionine (80 Ci/mmol) was obtained from New England Nuclear, and ^{14}C-reconstituted protein hydrolysate (21 Ci/mmol, manufacturer's mixture) was from Schwarz/Mann. Kanamycin sulfate and trimethoprim were purchased from Sigma, and tetracycline hydrochloride was from Calbiochem.

Bacterial Strains. *C. crescentus* CB15 spontaneous or Tn*5*-induced *fla* mutants were obtained from B. Ely (6, 19). SC512, SC520, SC1062, SC1133, and SC1121 all contain mutations (Fig. 1) that cause a flagella-minus phenotype. Mutant strain SC274 is a temperature-sensitive mutant with a partial flagellar structure (6, 12, 18). *E. coli* strain HB101 was obtained from Cold Spring Harbor Laboratories.

Plasmids. pRK290 and pRK2013 were obtained from G. Ditta (20). Plasmid pMP7 was constructed by ligating pBR325 and DNA from the *flaYE* gene cluster from *C. crescentus* SC1062 as described (15). Mutant SC1062 contains a Tn*5* insertion mutation in the *flaE* region of strain CB15 (Fig. 1*B*). DNA homologous to the CB15 *flaYE* region was isolated from a *C. crescentus* CB13 λWES clone bank (15) and then recloned into pBR325 to give the plasmid pA8 (Fig. 1*D*) and into pRK290 to give the plasmid pRB1 (Fig. 1*E*) by methods described by Davis *et al.* (21). Deletions of pRB1 (Fig. 1*E*) were constructed by digesting pRB1 DNA with *Xho* I or *Bam*HI, ligating with T4 DNA ligase, and then screening HB101 tetracycline-resistant transformants for plasmids of reduced size. Because pRK290 lacks *tra* (transfer) functions, the recombinant plasmids were mobilized from *E. coli* into *C. crescentus* by using a strain harboring pRK2013, which has *tra* functions intact but has a ColE1 replicon (20) that cannot replicate in *C. crescentus*.

Complementation. Plasmids were transferred from *E. coli* donor strains to *C. crescentus* recipients as described by Ely (22). In order to test for complementation, strains containing plasmids were purified twice on PYE (16) agar plates supplemented with tetracycline (3 μg/ml) and trimethoprim (30 μg/ml), and colonies were suspended in PYE medium and examined under the light microscope for ability to swim. Ability to carry out general chemotaxis was measured by streaking cells on semi-solid PYE plates (0.3% agar) containing tetracycline (3 μg/ml). Strains that are motile and generally chemotactic exhibit a broad, fuzzy streak; those which are not chemotactic (or which are nonmotile) show a thin streak with sharp boundaries.

RESULTS

Complementation. Several nonmotile, nonflagellated mutants that map within the *flaYE* gene cluster (Fig. 1*A*) were examined for their ability to be complemented by plasmids

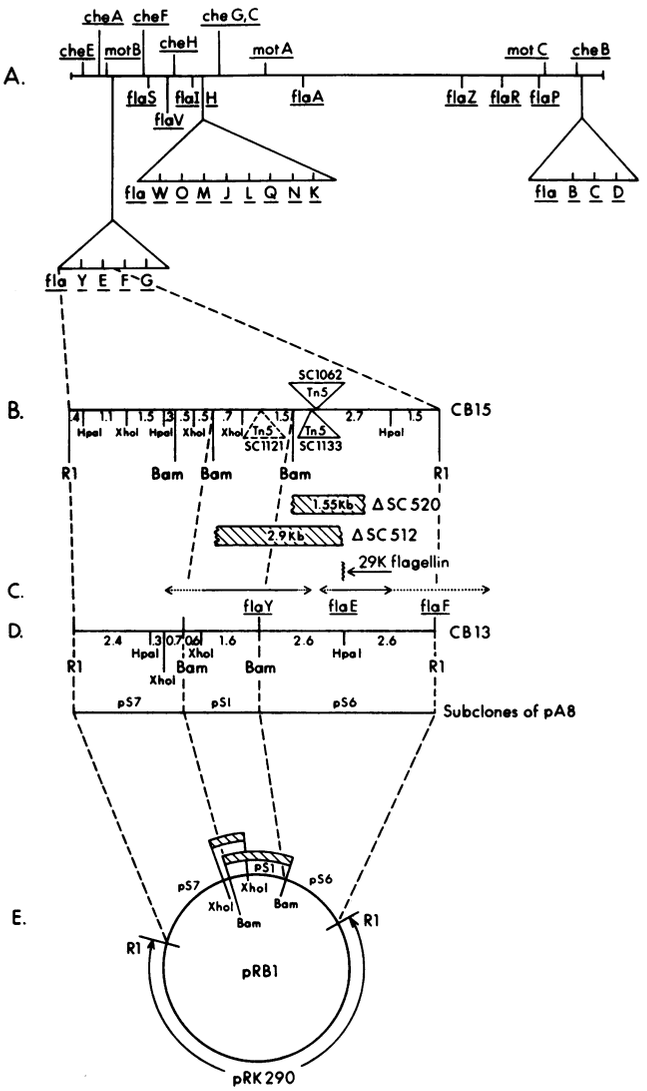

FIG. 1. (*A*) A partial map of the *C. crescentus* CB15 chromosome showing the relative location of known *fla*, *che*, and *mot* genes involved in flagellar biogenesis and function (refs. 6 and 7; B. Ely, personal communication). (*B*) Restriction map of the *flaYE* region of CB15 showing the location of Tn*5* insertions (shown as triangles) SC1121, SC1133, and SC1062 and deletions (shown as hatched bars below the restriction map) SC512 and SC520 (16). The *flaYE* region from SC1062 has been cloned in the plasmid pMP7 (16). (*C*) Genetic order and approximate locations of the *flaY* and *flaE* linkage groups. The location of *flaF* is to the right of the *Eco*RI (RI) restriction site. The location of the 29-kDa flagellin within *flaE* is as described by Gill and Agabian (14). (*D*) Restriction map of the homologous region from *C. crescentus* strain CB13 which has been cloned in the plasmid pA8 (16). Dashed lines connect subclones of pA8 (pS7, pS1, and pS6) to the homologous regions of the CB15 chromosome above and to the map of pRB1 in *E*. (*E*) Diagram of pRB1, which is comprised of the vector pRK290 and the pA8 insert. Hatched areas show regions deleted in the pRB1ΔXho and pRB1ΔBam plasmids.

containing cloned DNA from this region of the *C. crescentus* CB13 and CB15 chromosome. Mutations in each of the identified genes in this cluster cause loss of motility; *flaE* mutations yield a flagella-minus phenotype (6, 16), and *flaY* mutations result in the synthesis of a flagellum with a short filament composed of only a single flagellin, the 25-kDa species (refs. 6, 12, and 18; B. Ely, personal communication). The ability of the plasmid pRB1 (see Fig. 1*E*) to complement these mutants was tested by light microscopy (Table 1) and by observing streak morphology on semisolid plates. Motile cells capable of general chemotaxis yielded large, diffuse

Table 1. Methyltransferase and methyl-acceptor activity in mutant and in plasmid-containing strains

Strain	Methyl transferase activity,* pmol/mg per 30 min	Methyl-acceptor activity,† % of wild type	Swarm streak on soft agar	*In vivo* methylation‡
Wild-type CB15	20	100	+	+
SC274, pRK290	8	30	−	−
SC274, pRB1	13	50	+	+
SC512, pRK290	8	10	−	−
SC512, pRB1	15	50	+	+
SC520, pRK290	10	10	−	−
SC520, pRB1	18	50	+	+
SC1062, pRK290	9	20	−	−
SC1062, pRB1	21	75	+	+

*Membrane acceptor is from wild-type CB13 cells. Methyltransferase activity measures the incorporation of [^{3}H]methyl from *S*-adenosyl-[methyl-^{3}H]methionine as described (7).
†Methyl-acceptor activity was analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis as described (7).
‡See the legend to Fig. 3.

streaks. *flaE* deletions in SC512 and SC520, Tn*5* insertions in SC1133, SC1062, and SC1121, and the *flaY* temperature-sensitive mutation in SC274 were all complemented by pRB1, as measured by the width of the swarm on semisolid agar. None of the mutants were complemented by the pRB1 plasmids with *Xho* and *Bam* deletions shown in Fig. 1*E*. Larger *flaEF* deletions in SC507 and SC514, which extend rightward into *flaF* beyond the cloned region (16), and *flaG* and *pleB* mutations, which map outside and to the right of the cloned region (B. Ely, personal communication), were not complemented by pRB1.

Wild-type CB15 carrying either pRB1 or the deletion derivatives of pRB1 were motile and exhibited general chemotaxis. These results suggest that the presence of extra copies of this region of the genome are tolerated and that the deletion mutations constructed in the *flaY* region are recessive.

Several lines of evidence suggest that complementation by pRB1 is due to the presence of intact pRB1 and not due to recombination. The *C. crescentus* pRB1 DNA insert (pA8; see Fig. 1*D*) was nick-translated (23) and then hybridized to chromosomal and plasmid DNA prepared from each complemented strain. The chromosomal bands that showed homology to pA8 comigrated with bands present in each of the original deletion and insertion strains, and pA8 homology also was observed with resident plasmid DNA, which comigrated with pRB1. Additional evidence comes from the observation that all colonies of the complemented strains exhibited the same motile phenotype; if recombination were involved, it would not have occurred in all cases. Furthermore, strains containing deletion plasmids, which initially gave no evidence of complementation, yielded small flairs on the narrow streaks after many days of incubation on dilute agar. Similar flairs were not observed when the strain contained pRK290. Upon restreaking, these flairs gave a large diffuse streak. The occurrence of these flairs was at a much lower frequency than the rate of complementation and was probably the result of recombination. Recombination was confirmed in one case by the isolation of plasmid DNA from one such flair produced by SC1121/pRB1ΔXho. When the isolated plasmid DNA was transformed into *E. coli* and mated back into SC512 and SC1121, this plasmid restored the ability of the mutant strains to swim. The plasmid was found to have gained about 2 kilobases of DNA. Analysis of restriction digests showed the presence of a new *Hin*dIII site not originally present in pRB1. This *Hin*dIII site was mapped previously (16) to the CB15 DNA homologous to the region deleted in pRB1ΔXho. This shows that, in rare motile flairs, the CB13-derived deletion plasmid was able to pick up a homologous piece of the host chromosome.

Flagellin Synthesis. Several mutations in the region of the *flaYE* gene cluster 3′ to the flagellin structural genes (see Fig. 1*B*) result in low levels of synthesis of the 25-, 27.5-, and 29-kDa flagellins (16). In order to determine if the synthesis of these different flagellin species (which are immunologically crossreactive) was restored to wild-type levels in plasmid-containing strains, immunoprecipitates of labeled cell extracts were made with anti-flagellin antibody (Fig. 2). Autoradiography of immunoprecipitates separated on $NaDodSO_4$/polyacrylamide gels showed the wild-type pattern of flagellins from CB15. The presence of the pRB1 deletion plasmids, pRB1ΔXho and pRB1ΔBam, had little effect on this pattern, indicating that these deletion mutations were recessive to the wild type. With SC512 carrying pRK290, the flagellins were barely detectable, but both the 25- and 27.5-kDa proteins were restored in the presence of pRB1. There was a greater increase in the amount of the 27.5-kDa protein. Similar results were obtained with SC520 carrying pRB1 (Fig. 2). pRB1 plasmids with small ΔXho and ΔBam deletions (see Fig. 1*E*) partially restored the 27.5- and 25-kDa flagellins, but the magnitude of the increase was considerably less in the case of the 25-kDa flagellin. These plasmid deletions did not restore normal flagellar assembly and motility. Similar results were obtained with SC1062, SC1121, and SC1133. These results suggest that the region of *flaY* deleted in pRB1ΔXho and pRB1ΔBam plasmids is required for the normal expression of the flagellin proteins and motility.

The temperature-sensitive *flaY* mutant SC274 produced reduced levels of the 25- and 27.5-kDa flagellins and elevated levels of the 29-kDa protein at the restrictive but not at the

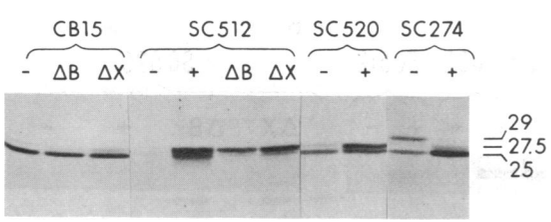

FIG. 2. Proteins immunoprecipitated by anti-flagellin antibody (24) from cell extracts of wild-type and mutant strains in the presence of various plasmids. Proteins (shown in kDa) were separated by electrophoresis through 12% $NaDodSO_4$/polyacrylamide gels and visualized by autoradiography. Wild-type *C. crescentus* strain AE5000 is shown as CB15, and *flaE* deletions SC512 and SC520 and the temperature-sensitive *flaY* SC274 are indicated, along with the presence of vector pRK290 alone (−), pRB1 (+), pRB1ΔBam (ΔB), or pRB1ΔXho (ΔX).

permissive temperature (Fig. 2). The presence of pRB1 restored motility at the restrictive temperature and caused an increase in the amount of the 25-kDa flagellin and a decrease in the production of the 29-kDa protein (Fig. 2). The presence of pRB1 also led to a decrease in the 29-kDa protein detected in SC1062, SC1121, and SC1133 (data not shown), implying that the relative proportions of the three flagellin species are regulated and may be required for normal filament assembly. A point mutation in *flaE* (SC519) failed to synthesize the 29-kDa flagellin, and the other two flagellins were produced at significantly reduced levels (data not shown).

Methylation of Chemotaxis Proteins. Methylation of the carboxyl side chains of integral membrane MCPs has been shown in *C. crescentus* to be catalyzed by a specific methyltransferase (7). Out of eight generally nonchemotactic mutants, one, *cheG*, lacks methyltransferase activity (7). Another gene, *cheC*, has been associated with the methylesterase protein (unpublished data). These genes are widely separated from the *flaYE* cluster, yet it was found that mutations present in strains SC274, SC512, SC520, and SC1062 exhibited reduced levels of methyltransferase activity (Table 1). Methyltransferase activity was restored to these strains when plasmid pRB1 was introduced. Membranes were isolated from these strains, and the ability of their MCPs to be methylated by methyltransferase from wild-type CB13 cells was measured (Table 1). The membranes isolated from SC274, SC512, SC520, and SC1062 containing the vector pRK290 had low levels of methyl-accepting ability that were partially restored by the presence of pRB1.

Methylation of the MCPs in mutant and plasmid-complemented strains was measured also *in vivo* (Fig. 3; Table 1). Cultures were labeled with [*methyl*-^{3}H]methionine, and the proteins were separated on polyacrylamide gels as described (7). Wild-type CB15 cultures exhibited several methylated membrane protein species that have been shown to be correlated with the cell's chemotactic ability (ref. 7; Fig. 3*A*). Mutants SC512, SC1062 (Fig. 3*A*), and SC520 (Fig. 3*B*) containing the vector pRK290 showed little or no methylation of these bands. In the presence of pRB1, the level of methylation increased significantly, confirming the results of the *in vitro* methylation experiments described above. Furthermore, the *flaY* mutant SC274 showed reduced *in vivo* methylation that was restored to wild-type levels in the presence of pRB1 (data not shown). *In vivo* methylation also was measured in the mutant strain SC520 containing either plasmid pRB1ΔXho or pRB1ΔBam (Fig. 3*B*). The deleted plasmids only partially restored the ability to methylate these MCPs *in vivo*.

These results show that the presence of the plasmid pRB1 is able to restore functions that are under the control of unlinked genes. By using deletion plasmids, a portion of the *flaY* region was shown to be essential for complementation. The restoration may be due to direct interaction of gene products from pRB1 with the genes for chemotaxis methylation, or it may be that the restoration of chemotaxis functions occurs subsequent to the restoration of flagellin synthesis and flagellar assembly allowed by pRB1.

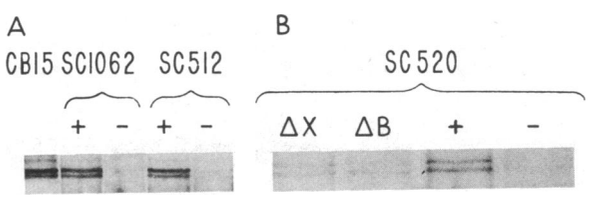

FIG. 3. *In vivo* [^{3}H]methylation of alkali-labile membrane proteins involved in chemotaxis. Cellular proteins were methylated in the absence of protein synthesis as described by Kort *et al.* (25) under the conditions as described (7). Cell extracts were made as described (7). Proteins were separated by electrophoresis through 10% $NaDodSO_4$/polyacrylamide gels and visualized by autoradiography. (*A*) Wild-type CB15 (AE5000) and mutants SC1062 and SC512 carrying either pRB1 (+) or pRK290 (−) as indicated. (*B*) SC520 containing the plasmids pRB1ΔXho (ΔX), pRB1ΔBam (ΔB), pRB1 (+), and pRK290 (−) as indicated.

DISCUSSION

The differential synthesis and release of a single localized flagellum during each *C. crescentus* cell division cycle has allowed an investigation of the regulatory interactions among the genes and gene products involved in localized flagellar biogenesis and the differential expression of chemotaxis functions. Some of us recently have demonstrated that at least three functions associated with the chemotaxis machinery, carboxymethyltransferase, the membrane MCPs, and a specific methylesterase, are differentially expressed coincident with the period of flagellar biogenesis and assembly at the cell pole (ref. 7; unpublished data). Furthermore, the membrane MCPs are synthesized prior to cell division, coincident with the synthesis of the flagellin and hook components of the flagellum, and are localized specifically in the membrane of the insipient swarmer cell (ref. 7; unpublished data). Upon cell division, the resulting stalked cell lacks MCPs, whereas the swarmer cell contains these proteins along with a flagellum. Methyltransferase activity is similarly detected only in the swarmer cell upon cell division (ref. 7; unpublished data). These results suggest that the localization of both the flagellum and the proteins involved in chemotaxis at the swarmer cell pole of the dividing cell may present physical constraints on their order of assembly at the cell surface.

Here we show that mutations that map in the *flaYE* gene cluster (16), 3′ to flagellin structural genes (14, 16) and separate from identified genes involved in chemotaxis methylation (7), prevent flagellar assembly and decrease the expression of several flagellin structural genes and chemotaxis methylation genes. As has been suggested for *E. coli* (3), it appears that in *C. crescentus* a hierarchy of flagellar and chemotaxis gene expression may function as a control mechanism to coordinate the order of synthesis of specific proteins and the location of their assembly.

In order to dissect the regulatory functions in this *flaYE* region and to determine whether they could function in *trans* to regulate other *fla* and *che* genes, we constructed plasmids containing CB13 DNA which hybridized to this region of the CB15 chromosome. The presence of the plasmid pRB1, containing an intact *flaYE* region, allowed swimming and chemotaxis in several nonmotile strains containing mutations in the *flaYE* region but not in any nonmotile strains that had mutations outside the cloned region. Specifically, the presence of pRB1 resulted in (*i*) an increase in the amount of the two major components of the flagellar filament, 27.5- and 25-kDa flagellins, (*ii*) a decrease in the minor 29-kDa flagellin, (*iii*) an increase in methyltransferase and methyl-acceptor activity measured by *in vitro* reconstitution experiments, and (*iv*) an increase in *in vivo* carboxymethylation of membrane methyl-acceptor proteins. Because a second flagellin gene cluster is known to exist at another location on the *C. crescentus* chromosome (14), the "complemented" expression of the 25- and 27.5-kDa flagellin proteins in the presence of pRB1 may reflect increased expression of unlinked chromosomal flagellin genes as well as expression from the plasmid.

Every mutant strain that became motile when they harbored pRB1 was unable to swim in the presence of the plasmids pRB1ΔXho or pRB1ΔBam, which were deleted in the *flaY* region 3′ to the flagellin structural genes (see Fig. 1*E*) and were unable to assemble an intact flagellum, although

they appeared to restore the 27.5-kDa flagellin and to partially restore both the 25-kDa flagellin and chemotaxis methylation. The fact that none of the mutations in the *flaE* region was fully complemented by the *flaY* deletion plasmids has two possible explanations. It may be that the *flaE* mutations tested have polar effects on or include *flaY*, or that on the plasmid the *flaY* deletions are polar with respect to *flaE*. Alternatively, it may be that both regions must be intact on the same piece of DNA in order to be functionally expressed.

The third flagellin-like protein of 29-kDa, which may function transiently in flagellar filament assembly or may be a very minor component of the filament (14), was not detected in the SC512 and SC520 strains containing deletion mutations (see Fig. 1*A*; refs. 16 and 17). Complementation of these mutants with pRB1 failed to restore detectable synthesis of the 29-kDa flagellin, although both the 25- and 27.5-kDa flagellins were restored and a functional filament was assembled. Furthermore, the level of the 29-kDa flagellin detected in the mutants SC274, SC1062, SC1121, and SC1133 was decreased in the presence of pRB1, and pRB1 restored normal filament assembly and motility to these mutants. These results suggest that the relative proportion of the 29-kDa protein to the other flagellins is regulated and that its overproduction may interfere with the normal assembly of the filament.

FlaYE deletions, Tn*5* insertions, and a temperature sensitive *flaY* mutant had low levels of MCP methylation. Because the genes required for MCP methylation and chemotaxis functions reside elsewhere on the chromosome (7), these results suggest that the *flaYE* region encodes proteins that function in *trans* to regulate the expression of chemotaxis genes. Recently Gill and Agabian (14) have determined the sequence of the 29-kDa flagellin gene that resides within the *flaE* region of the CB15 chromosome (see Fig. 1*C*). Our results demonstrate that sequences 3′ to this flagellin gene function to regulate the expression of genes involved in chemotaxis methylation either directly or by a regulatory cascade requiring the successive expression of several flagellar genes. The *flaY* region appears to encode at least one protein because mutant SC274 (*flaY*) is temperature sensitive for flagellin assembly (6, 12). The *flaY* gene product, shown to be required for regulating the level of flagellin expression and flagellar assembly, may be a component of the flagellar structure whose synthesis is part of a regulatory hierarchy that includes the genes required for chemotaxis methylation.

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