JOURNAL OF BACTERIOLOGY, Sept. 1997, p. 5849–5853 Vol. 179, No. 18
0021-9193/97/$04.00+0

Roles of the Histidine Protein Kinase PleC in *Caulobacter crescentus* Motility and Chemotaxis

GEORGE J. BURTON, GREGORY B. HECHT,† AND AUSTIN NEWTON*

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

Received 3 February 1997/Accepted 11 July 1997

The *Caulobacter crescentus* histidine kinase genes *pleC* and *divJ* have been implicated in the regulation of polar morphogenesis and cell division, respectively. Mutations in *pleC* also potentiate the cell division phenotype of *divJ* mutations. To investigate the involvement of the PleC kinase in motility and cell cycle regulation, we carried out a pseudoreversion analysis of the *divJ332* allele, which confers a temperature-sensitive motility (Mot$^-$) phenotype. All cold-sensitive pseudorevertants with a Mot$^+$ phenotype at 37°C and a cold-sensitive swarm phenotype in soft agar at 24°C contained extragenic suppressors that were null mutations mapping to *pleC*. Instead of a cell division defect at the nonpermissive temperature, however, revertants displayed a cold-sensitive defect in chemotaxis (Che$^-$). In addition, the mutant cells were also supermotile, a phenotype previously associated only with mutations in the response regulator gene *pleD* that block the loss of motility. We also found that the Mot$^-$ defect of *pleC* mutants is suppressed by a *pleD301*/*pleD*$^+$ merodiploid and results in a similar, supermotile, cold-sensitive Che$^-$ phenotype. These results implicate signal transduction pathways mediated by PleC-DivK and DivJ-PleD in the regulation of chemotaxis as well as motility. We discuss these findings and the observation that although the PleC kinase does not play an indispensable role in cell division, a temperature-sensitive allele of *pleC* (*pleC319*) has severely reduced viability under stringent growth conditions.

Caulobacter crescentus is an aquatic bacterium that divides asymmetrically to generate two distinct daughter cells, a nonmotile stalked cell and a motile swarmer cell. The differentiated stalked cell gives rise to a new swarmer cell at the end of each cell cycle as a result of precisely ordered developmental events at the stalk-distal pole of the dividing cell. Differentiation of this pole begins with the assembly of a single flagellum, which begins to rotate just prior to cell separation. Receptors for polar bacteriophages are also formed at the flagellar pole, and following cell separation, the developmental sequence continues in the new swarmer cell with the assembly of polar pili, loss of motility and shedding of the flagellum, and formation of the stalk (reviewed in references 3, 6, and 14).

Production of a motile, chemotactic swarmer cell at division depends on the strict temporal regulation of developmental events. Genetic analysis of the pleiotropic developmental gene *pleC* has identified genes encoding members of two-component signal transduction pathways (11, 19) responsible for the coordination of differentiation and cell cycle regulation in *C. crescentus* (reviewed in reference 16). Suppressors of *pleC* mutations mapping to *divJ*, *divK*, and *divL* confer a conditional cell division phenotype (24), while suppressors in *pleD* divide normally and assemble functional flagella but never lose motility and thus display a supermotile or Mot* phenotype (10, 23).

Both *pleC* (26) and *divJ* (15) encode histidine protein kinases, while *divK* encodes an essential, single-domain response regulator (8) that belongs to the CheY family of proteins (25). The *pleD* gene product is predicted to be a compound response regulator that includes a unique C-terminal GGDEF domain (10). Genetic and biochemical analyses have provided strong evidence that PleC and DivK form a cognate kinase-response regulator pair required to turn on flagellum rotation and that DivK plays a second, essential role early in the cell cycle to initiate cell division, apparently in response to the DivJ kinase (8, 16). Another essential response regulator, CtrA, is now known to function directly in cell cycle regulation (21), and a recent genetic study suggests that DivJ and DivK are members of a signal transduction pathway regulating activity of this transcription regulator (17).

Despite evidence for separate signal transduction pathways regulating motility (PleC ➡ DivK) and the initiation of cell division (DivJ ➡ DivK), the catalytic domains of both DivJ and PleC catalyze the phosphorylation of DivK and the dephosphorylation of phospho-DivK in vitro (8). It is also known that some *pleC* alleles potentiate the division defect conferred by cold-sensitive *divJ* mutations (24). These results suggested that *pleC* might play a role in the regulation of both cell division and motility. To examine this possibility more closely, we have carried out a genetic analysis of the *divJ322* strain, which displays a partial cold-sensitive cell division defect and a temperature-sensitive defect in motility (Mot^{Ts}) (24). In these studies, we have isolated cold-sensitive pseudorevertants of the *divJ322* strain that form swarms (Swm$^+$) in a semisolid medium at 37°C but not at 24°C. Defects in cell division, as well as motility, can be detected in this screen because the movement of filamentous cells is restricted in a soft agar.

Our results show, first, that the cold-sensitive phenotype of the 15 strains examined resulted from a defect in chemotaxis (Che$^-$) at 24°C rather than a defect in motility (Mot$^-$) or cell division and, second, that the suppressor mutations in these pseudorevertants were null alleles in *pleC* that also conferred an unconditional supermotility phenotype in the *divJ332* background. Suppression of the *pleC* motility defect by a *pleD301*/*pleD*$^+$ merodiploid resulted in a similar, supermotile, Che$^-$

* Corresponding author. E-mail: anewton@molecular.princeton.edu.

† Present address: Department of Biological Sciences, Rowan University, Glassboro, NJ 08028.

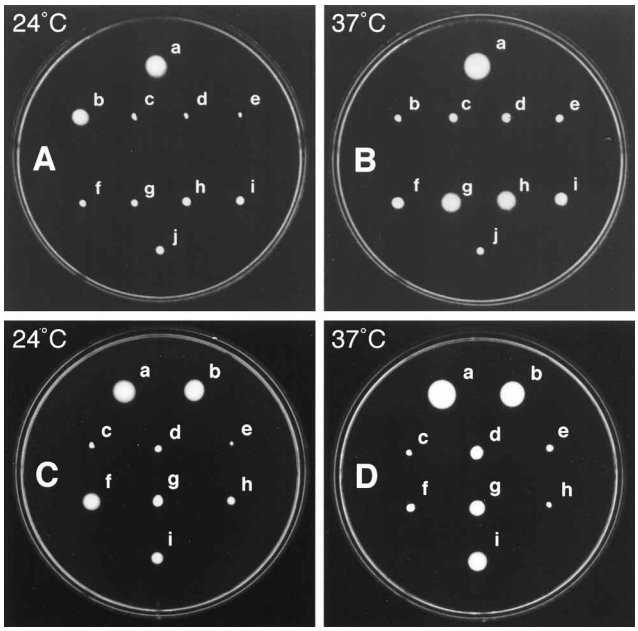

FIG. 1. Assays of swarming ability in semisolid agar plates. (A and B) Single colonies of *C. crescentus* strains PC5347 (*divJ*$^+$ *pleC*$^+$) (a), PC5300 (*pleC319*) (b), PC9192 (*pleC362*) (c), PC9154 (*pleC371*::Tn*5*) (d), PC4253 (Δ*pleC376::aacC1*) (e), PC5332 (*divJ332 pleC319*) (f), PC9107 (*divJ332 pleC362*) (g), PC9135 (*divJ332 pleC371*::Tn*5*) (h), PC4254 (*divJ332* Δ*pleC376::aacC1*) (i), and PC5353 (*divJ332*) (j) were stabbed into 0.35% agar plates made with PYE medium plus 50 μg of kanamycin per ml which were incubated for 2 days at 24°C (A) or 37°C (B). *pleC362* is a suppressor of *divJ332* that behaves as a point mutation; *pleC371*::Tn*5* is another *divJ* suppressor that contains both a Tn*5* insertion and a deletion of a portion of the gene (see text for details). (C and D) Assay of swarming ability of *pleD* merodiploid strains. Single colonies of *C. crescentus* strains CB15 (*pleC*$^+$ *pleD*$^+$) (a), CB15::pGH503 (*pleC*$^+$ *pleD*$^+$/*pleD301*) (b), PC5225 (*pleC301*::Tn*5 pleD*$^+$) (c), PC8993 (*pleC301*::Tn*5 pleD301*) (d), PC5225::pGH503 (*pleC301*::Tn*5 pleD*$^+$/*pleD301*) (e), PC5262 (*pleC319 pleD*$^+$) (f), PC4166 (*pleC319 pleD301*) (g), PC5262::pGH503 (*pleC319 pleD*$^+$/*pleD301*) (h), and PC4707 (*pleC319 pleD302::kan*) (i) were stabbed into 0.35% agar plates made with PYE medium which were incubated for 2 days at 24°C (C) or 37°C (D). pGH503 is a nonreplicating, integrative plasmid carrying the *pleD301* allele.

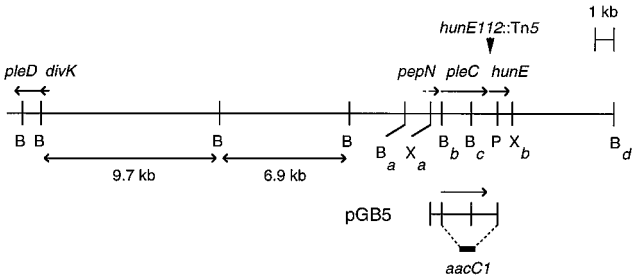

FIG. 2. Genome organization of the *pleC-divK-pleD* region in *C. crescentus*. The top line shows a partial restriction map of the *pleC-divK-pleD* region of the chromosome; arrows denote the direction of transcription of *pleD* (10), *divK* (8), the 3′ fragment of *pepN* (an aminopeptidase N homolog), *pleC*, and the 1.0-kb ORF downstream of *pleC* (26). The location of the *hunE112*::Tn*5* insertion is indicated by an arrowhead. This insertion was determined by Southern blotting to lie between the 3′ end of *pleC* and the *Sal*I site at nucleotide 201 of the 1.0-kb ORF (data not shown and reference 26). Thus, it is likely that the 1.0-kb ORF corresponds to the *hunE* gene. The extent of the insert in complementing clone pGB5 is shown below the partial restriction map, and the region replaced in the Δ*pleC376::aacC1* allele is also shown. Abbreviations: B, *Bam*HI; P, *Pst*I; X, *Xho*I. Not all *Pst*I and *Xho*I sites are necessarily shown.

phenotype. These results implicate signal transduction pathways mediated by PleC-DivK and DivJ-PleD in the regulation of chemotaxis as well as motility.

MATERIALS AND METHODS

Strains and growth conditions. All *C. crescentus* strains used in this study were derivatives of wild-type strain CB15 (ATCC 19089); they were routinely grown at 30°C in rich PYE medium (20). Solid medium consisted of either PYE broth or M2 minimal salts containing 0.2% glucose (12) plus 2% agar. Motility agar contained 0.30 or 0.35% agar in 0.5× PYE (18). *Escherichia coli* MC1061 and HB101 were used as hosts for cloning and conjugations, and strain XL-1 Blue (Stratagene) was used as a host for cloning into vector pBluescript I KS(+) (Stratagene). *E. coli* strains were grown at 37°C in ML medium (13, 18) or on solid medium consisting of ML medium plus 2% agar. Media were supplemented where indicated with antibiotics at the following concentrations: kanamycin at 50 μg/ml in PYE and ML media; tetracycline at 2 μg/ml in M2 and PYE media and at 25 μg/ml in ML medium; ampicillin at 50 μg/ml in ML medium, and gentamicin at 2 μg/ml in PYE medium.

Isolation of suppressors of *divJ332*. Individual colonies of strain PC5353, which carries the *divJ332* allele and the *hunE112*::Tn*5* marker ca. 88% linked to the *pleC* locus, were stabbed into semisolid PYE agar and incubated at 37°C. Spontaneous revertants were picked from the individual swarming flares, streaked onto PYE plates, and incubated at 37°C. Three single colonies of each revertant isolate were inoculated into motility agar, grown in parallel at 24 and 37°C, and examined for cold-sensitive defects in cell division and swarming.

Mapping of suppressors. The revertants of *divJ332* were screened for suppressors in the *pleC* region by preparing bacteriophage φCr30 lysates (5) on these isolates and transducing strain CB15. Recombinants containing the *hunE*::Tn*5* marker were selected on PYE-kanamycin plates at 30°C and screened for a linked nonswarming phenotype in semisolid agar containing kanamycin at 24, 30, and 37°C.

Phenotypic assays. Swarming was assayed by stabbing individual colonies into semisolid agar and scoring swarm size after 2 days of growth at the appropriate temperature. For motility and cell division phenotypes, an overnight culture grown at the permissive temperature was subcultured by dilution of 1:40 into fresh PYE medium and examined for motility and cell defects in the mid-log phase by phase-contrast microscopy. Percentages of motile cells were determined by scoring cell movement in similar cultures that had been recorded on videotape. Stalk formation was assayed by electron microscopy, and resistance to bacteriophage φCbK was determined by cross-streaking on PYE plates (23).

Construction of *pleC* replacement allele. The 3.2-kb *Bam*HIb-*Pst*I fragment, which includes the entire *pleC* gene, was replaced with the *aacC1* Gm^r cassette (22) in the 4.5-kb *Xho*Ia-*Xho*Ib insert of pBluescript I KS(+)-derived plasmid pGH534 (7). The replacement allele and flanking chromosomal DNA (see Fig. 2 below) were subcloned into nonreplicating, integrative Tc^r plasmid pGH500 (8). The resulting plasmid pGB29 was transferred to wild-type strain CB15 by conjugation and integrated at the *pleC* locus by selection for Tc^r recombinants, and the gene replacement was isolated as described previously (8). The presence of the cassette at the expected location on the chromosome was confirmed by Southern blotting.

Colony formation assay. Single colonies from either fresh PYE or M2 plates were inoculated into the same liquid medium, grown overnight at 24°C, subcultured by dilution of 1:40 into the same medium, and grown at 24°C for 9 h. The absorbance of each subculture at 650 nm (A_{650}) was determined, and the CFU were determined in duplicate on plates containing the same medium. Viability was expressed as CFU per milliliter per A_{650}.

TABLE 1. Percentage of motile cells in cultures of *pleC* and *divJ* mutant strains

Strain	Relevant genotype	% of motile cells at[a]:		
		24°C	30°C	37°C
CB15	Wild type	9.4	9.1	10
PC5225	*pleC301*::Tn*5*	<2	<2	<2
PC4166	*pleC319 pleD301*	30	52	66
PC5353	*divJ332*	15	1.2	1.4
PC9107	*divJ332 pleC362*	15	30	21
PC9111	*divJ332 pleC364*	19	25	20
PC9119	*divJ332 pleC366*	13	18	26
PC9124	*divJ332 pleC368*	21	23	28
PC8985	*divJ332 pleC301*::Tn*5*	30	61	15
PC8986	*divJ332 pleC302*::Tn*5*	30	42	23

[a] Percentage of single cells recorded on videotape exhibiting motility in liquid cultures (see Materials and Methods).

RESULTS AND DISCUSSION

Isolation of *pleC* alleles that suppress *divJ332*. The *divJ332* strain PC5353 displays a Mot^- phenotype at 37°C and, consequently, forms tight swarms (Swm^-) on semisolid agar plates at this temperature (Fig. 1B, colony j). The inability of this strain to form swarms at 24°C (Fig. 1A, colony j) may be attributed to the cell division defect described previously for this strain (24). We initially isolated 40 spontaneous motile revertants able to swarm in semisolid medium at 37°C and then screened for the formation of tight swarms on the soft agar plates at 24°C, expecting to identify mutants with cold-sensitive defects in either cell division or motility (Fig. 1A and B, colonies g and h). Fifteen of the 16 cold-sensitive revertants from the initial screen contained second-site suppressors that mapped to the *pleC-divK-pleD* gene cluster, as shown by their 68 to 100% linkage to the *hunE112*::Tn*5* insertion (Fig. 2). These extragenic suppressors were mapped to *pleC* by complementation with plasmid pGB5, which contains an intact *pleC* gene (Fig. 2) (26). None of the remaining 24 Swm^+ revertants displayed a conditional phenotype or displayed genetic linkage to *pleC* (4).

***divJ pleC* strains display a cold-sensitive defect in chemotaxis and a supermotile phenotype.** Observation of the *divJ322-pleC* pseudorevertants by phase-contrast microscopy revealed that a substantial number of the cells were motile in liquid cultures at 24°C as well as at 30 and 37°C (Table 1); few filamentous cells were observed at any of the three temperatures. Thus, none of the suppressor alleles conferred a defect in cell division at 24°C, and we concluded that the conditional swarm phenotype of the pseudorevertants in semisolid medium (Fig. 1A, colonies g and h) is due primarily to a cold-sensitive defect in chemotaxis. A small number of suppressors of the *divJ322* Mot^- phenotype had been isolated previously at 37°C and mapped to *pleC* (24), but these revertants were not examined at 24°C in soft agar plates for swarm formation, and consequently, conditional chemotactic defects would not have been observed. The supermotile phenotype described below was also not reported for these revertants.

An unexpected phenotype of the *divJ322-pleC* pseudorevertants observed by light microscopy was the presence of motile cells at all stages of the cell division cycle in liquid cultures. We confirmed these visual observations by videotaping mid-log-phase cultures of four *divJ332 pleC* strains grown at 24, 30, or 37°C (see Materials and Methods). The percentages of motile cells at 30°C (18 to 30%) were ca. two- to threefold higher than those in a culture of wild-type strain CB15 at the same temperature (Table 1), compared to <2% motile cells in the parental *divJ332* strain PC5353 at 30 and 37°C. Electron microscopy of cultures of revertant strains PC9107 (*divJ332 pleC362*) and PC9111 (*divJ332 pleC364*) revealed that they contained stalkless, biflagellated cells (4), which is characteristic of supermotile *pleD* strains (10, 23). Only *pleD* mutants, which are defective in loss of motility and stalk formation (10, 23), have been reported previously to exhibit this supermotile or Mot* phenotype.

Characterization of suppressor alleles in *pleC*. When the *pleC* suppressor mutations were back-crossed into a wild-type, $divJ^+$ genetic background and examined by light microscopy, all of the *pleC* strains divided normally and all but one were nonmotile and resistant to bacteriophage φCbK at 24, 30, and 37°C (4). The *pleC* strains formed very tight swarms in semisolid agar (Fig. 1A and B, swarms c and d), and the *pleC362* and *pleC364* mutant strains displayed the characteristic stalkless, multiply flagellated phenotypes by electron microscopy (4).

Marker rescue experiments and physical mapping by Southern blotting demonstrated that four of the suppressors in *pleC* arose as rearrangements in which the Tn*5* in the adjacent *hunE* gene (Fig. 2) transposed into the 3′ sequence of *pleC* encoding the catalytic domain of the protein. The extent of these rearrangements suggested that the *pleC* suppressor alleles are null mutations which are responsible for suppressing the temperature-sensitive motility phenotype of *divJ332*. We confirmed this conclusion by constructing a *pleC* allele in which the entire open reading frame (ORF) was replaced with the gentamicin resistance cassette *aacC1* of *Pseudomonas aeruginosa* (22) (see Materials and Methods) (Fig. 2). The Δ*pleC376::aacC1* replacement allele in strain PC4253 produced a nonmotile, phage φCbK-resistant phenotype in an otherwise-wild-type background. It also suppressed the temperature-sensitive motility defect of *divJ332*, giving a supermotile, cold-sensitive chemotactic phenotype in the mutant *divJ* background on semisolid agar (Fig. 1A and B, swarms e and i). This result and the physical mapping experiments considered above indicate that the suppressor mutations mapping to *pleC* are probably null alleles.

The *pleC* disruption mutant did not exhibit defects in cell division when grown at 24 or 37°C in PYE medium, confirming that *pleC* is not essential for viability, as suggested by the isolation of Tn*5* insertions within *pleC* (23, 26). Thus, although PleC is a cognate histidine kinase for DivK, which is essential for cell division control (8), these results are consistent with a model in which PleC is normally involved only in the regulation of motility (see Fig. 4). When grown in M2 minimal medium, the *pleC* disruption mutant formed some short filaments at 37°C (4) and its plating efficiency on minimal medium at 37°C was ca. 60% of that of the wild-type strain. However, the Mot^{Ts} *pleC319* strain PC5262 displayed a plating efficiency on minimal medium at 37°C of only 3% of the wild-type level (see Materials and Methods). The *pleC319* strain also displayed a pronounced cell division defect under these conditions, with many of the cells forming long filaments (Fig. 3D). These results suggest that the *pleC319* allele is not a null mutation at the nonpermissive temperature and raise the possibility that under some growth conditions, the PleC kinase plays a role in timing or coordinating growth and cell division. We think it more likely, however, that the defective PleC protein produced at 37°C by the Mot^{Ts} *pleC319* mutant forms an abortive complex with DivK or has an altered catalytic activity toward this essential response regulator (8) and, as a consequence, interferes with the normal DivJ-DivK interaction required for cell division (Fig. 4). This would explain why some point mutations like *pleC319*, which maps within the N-terminal portion of PleC (4), potentiate the cell division defect of conditional *divJ* alleles, while the *pleC* null mutations examined here do not.

Signal transduction pathways regulating motility and chemotaxis. As modeled in Fig. 4, pseudoreversion studies have suggested that the histidine kinase PleC and response regulator DivK mediate a signal transduction pathway responsible for turning on motility late in the cell cycle (8). PleD acts in a second signal transduction pathway as a negative regulator responsible for turning motility off during the swarmer-to-stalked-cell transition, possibly in response to the DivJ kinase (10). The novel Che^- phenotype displayed by the *divJ332 pleC* pseudorevertants (Fig. 1; Table 1) suggested to us that these two pathways might also function in the regulation of chemotaxis (Fig. 4).

Support for this possibility comes from experiments with *pleD* mutant alleles and merodiploid strains. The *pleD301* mutation suppresses the Mot defect of a Tn*5* insertion in *pleC*

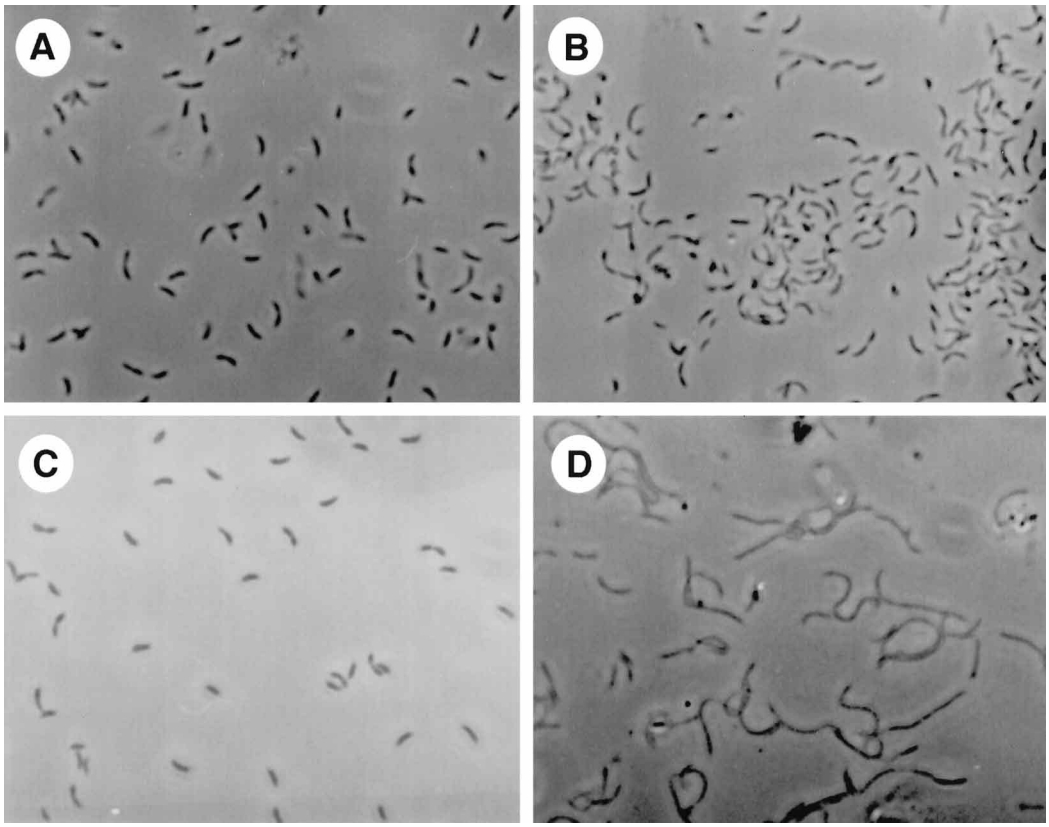

FIG. 3. Cell division phenotypes of a *pleC319* mutant in minimal medium. Overnight cultures grown at 30°C in M2 minimal medium were diluted 40-fold into the same medium and grown to the mid-exponential phase at 24 and 37°C. The cells were fixed by the addition of glutaraldehyde to a concentration of 0.5%, mounted on polylysine-coated slides, and examined by phase-contrast microscopy. (A and B) CB15 (wild type) at 24 and 37°C, respectively; (C and D) PC5262 (*pleC319*) at 24 and 37°C, respectively.

(*pleC301*::Tn*5*) and confers a Mot* (supermotile), Che^+ phenotype, as shown for strain PC8993 (Fig. 1C and D, swarm d) (10). The Mot* Che^+ phenotype of *pleD* mutants is thought to result from the failure of these cells to inhibit motility and chemotaxis during G_1 and effectively to bypass the requirement of PleC for turning on chemotaxis (Fig. 4). (PleD could also function in the G_2 period, as discussed below.) Introduction of the *pleD301* allele on plasmid pGH503 into the *pleC301*::Tn*5* strain to generate the *pleD301*/*pleD*$^+$ merodiploid (PC5225::pGH503) also suppressed the Mot^- defect of this *pleC* mutation in liquid cultures (9) and resulted in the Mot* phenotype. The strain was defective in chemotaxis, however, as indicated by the formation of small swarms in semisolid agar (Fig. 1C and D, swarm e). In similar experiments, the *pleD301* mutation suppressed the Mot^{Ts} allele *pleC319* to give a Mot* Che^+ phenotype at the nonpermissive temperature of 37°C (Fig. 1C and D, swarm g). The *pleC319 pleD*$^+$/*pleD301* merodiploid strain (PC5262::pGH503) displayed the Mot^+ phenotype in liquid cultures, but the formation of small swarms by this strain at 37°C in soft agar (Fig. 1D, swarm h) is consistent with a Che^- phenotype.

These results indicate that motility but not the Che^- defect of *pleC* mutants is suppressed in the *pleD*$^+$/*pleD301* merodiploid strains, and they implicate the response regulator PleD in the regulation of chemotaxis. Evidence that *divK* may also be involved in the regulation of chemotaxis came from experiments with the *divK341* mutation, which is known to restore motility and chemotaxis to *pleC*::Tn*5* mutants (8). However, a *pleC*::Tn*5* *divK341*/*divK*$^+$ merodiploid is motility positive but chemotaxis negative when assayed with soft agar plates (7), a situation similar to that observed with the *pleD* merodiploid strains above. The motility of *divJ332 pleC* (null) strains apparently depends on the activity of the mutant DivJ332 kinase (note that *divJ*$^+$ *pleC* null strains are Mot^-). Consequently, we suggest that the DivJ332 protein may be responsible for the abnormal regulation of PleD and/or DivK and the observed Che^- Mot* defects in *pleC divJ332* pseudorevertant strains.

Taken together, these results implicate the two signal transduction pathways mediated by PleC-DivK and DivJ-PleD in the regulation of motility and chemotaxis, as diagrammed in Fig. 4. Although these two pathways are assumed to function during the G_2 and G_1 periods, respectively (Fig. 4), PleC could also act directly on PleD during the G_2 period to inhibit the negative regulatory role of PleD in chemotaxis, as has been proposed previously for the function of PleD in the regulation of motility (10). How PleD and DivK might control chemotaxis is unclear. McpA, a methyl-accepting protein in *C. crescentus*, is synthesized only in predivisional cells during the G_2 period (1), and the proteolysis of McpA occurs in the G_1 period during the swarmer-to-stalked-cell transition (2). If the mutant signal transduction pathways examined in the present study have a direct effect on the chemotactic apparatus, we speculate that it might be apparent in the regulation of *mcpA* transcription or the stability of chemotaxis proteins.

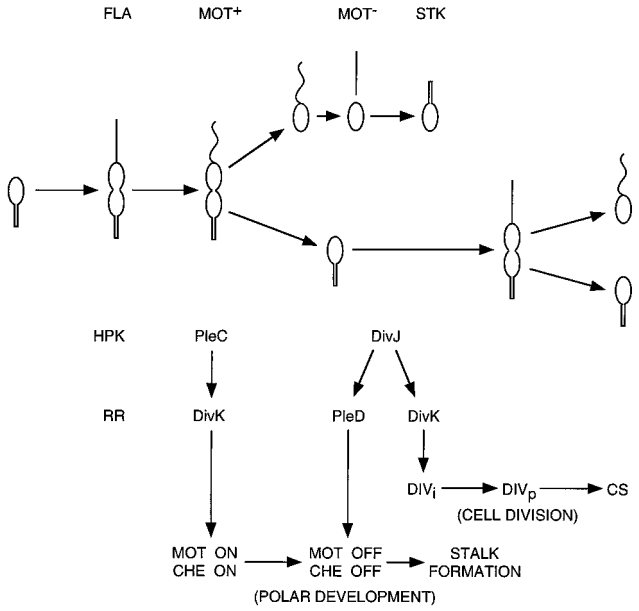

FIG. 4. Model for signal transduction pathways regulating cell division, motility, and chemotaxis in *C. crescentus*. This model proposes that the response regulator (RR) DivK acts at two different times in the cell cycle to control polar morphogenesis and cell division. Late in the cell cycle, DivK responds to the PleC histidine protein kinase (HPK) to initiate motility, chemotaxis, and stalk formation, and early in the next cell cycle, it responds to another sensor HPK, shown here as DivJ, to regulate cell division. PleD is predicted to control the loss of motility and chemotaxis during G_1; PleD appears to be regulated by the DivJ kinase. For details, see the text. Abbreviations: FLA, flagellum biosynthesis; MOT, motility; CHE, chemotaxis; STK, stalk formation; DIV_i, division initiation; DIV_p, division progression; CS, cell separation.

ACKNOWLEDGMENTS

We thank Todd Lane for preparation of phase-contrast micrographs and Noriko Ohta for assistance in the initial cloning of *pleC* and her critical reading of the manuscript.

This work was supported in part by research grant VM-46 from the American Cancer Society and by Public Health Service predoctoral training grant GM07388 to G.J.B. and G.B.H. from the National Institutes of Health.

REFERENCES

1. **Alley, M. R. K., S. L. Gomes, W. Alexander, and L. Shapiro.** 1991. Genetic analysis of a temporally transcribed chemotaxis gene cluster in *Caulobacter crescentus*. Genetics **129:**333–342.
2. **Alley, M. R. K., J. R. Maddock, and L. Shapiro.** 1993. Requirement of the carboxyl terminus of a bacterial chemoreceptor for its targeted proteolysis. Science **259:**1754–1757.
3. **Brun, Y. V., G. Marczynski, and L. Shapiro.** 1994. The expression of asymmetry during *Caulobacter* cell differentiation. Annu. Rev. Biochem. **63:**419–450.
4. **Burton, G. J., and A. Newton.** Unpublished data.
5. **Ely, B., and R. C. Johnson.** 1977. Generalized transduction in *Caulobacter crescentus*. Genetics **87:**391–399.
6. **Gober, J. W., and M. V. Marques.** 1995. Regulation of cellular differentiation in *Caulobacter crescentus*. Microbiol. Rev. **59:**31–47.
7. **Hecht, G. B.** 1995. Control of cellular differentiation in *Caulobacter crescentus* by signal transduction. Ph.D. thesis. Princeton University, Princeton, N.J.
8. **Hecht, G. B., T. Lane, N. Ohta, J. M. Sommer, and A. Newton.** 1995. An essential single domain response regulator required for normal cell division and differentiation in *Caulobacter crescentus*. EMBO J. **14:**3915–3924.
9. **Hecht, G. B., and A. Newton.** 1994. Unpublished results.
10. **Hecht, G. B., and A. Newton.** 1995. Identification of a novel response regulator required for the swarmer-to-stalked-cell transition in *Caulobacter crescentus*. J. Bacteriol. **177:**6223–6229.
11. **Hoch, J. A., and T. J. Silhavy.** 1995. Two-component signal transduction. ASM Press, Washington, D.C.
12. **Johnson, R. C., and B. Ely.** 1977. Isolation of spontaneously derived mutants of *Caulobacter crescentus*. Genetics **86:**25–32.
13. **Mullin, D. A., S. M. VanWay, C. A. Blankenship, and A. H. Mullin.** 1994. FlbD has a DNA-binding activity near its carboxy terminus that recognizes *ftr* sequences involved in positive and negative regulation of flagellar gene transcription in *Caulobacter crescentus*. J. Bacteriol. **176:**5971–5981.
14. **Newton, A. and N. Ohta.** 1990. Regulation of the cell division cycle and differentiation in bacteria. Annu. Rev. Microbiol. **44:**689–719.
15. **Ohta, N., T. Lane, E. G. Ninfa, J. M. Sommer, and A. Newton.** 1992. A histidine protein kinase homologue required for regulation of bacterial cell division and differentiation. Proc. Natl. Acad. Sci. USA **89:**10297–10301.
16. **Ohta, N., and A. Newton.** 1996. Signal transduction in the cell cycle regulation of *Caulobacter* differentiation. Trends Microbiol. **4:**326–332.
17. **Ohta, N., and A. Newton.** Evidence for a phosphorelay controlling cell division and development in *Caulobacter*, abstr. H-160, p. 311. *In* Abstracts of the 97th General Meeting of the American Society for Microbiology 1997. American Society for Microbiology, Washington, D.C.
18. **Ohta, N., E. Swanson, B. Ely, and A. Newton.** 1984. Physical mapping and complementation analysis of transposon Tn*5* mutations in *Caulobacter crescentus*: organization of transcriptional units in the hook gene cluster. J. Bacteriol. **158:**897–904.
19. **Parkinson, J. S.** 1993. Signal transduction schemes of bacteria. Cell **73:**857–871.
20. **Poindexter, J. S.** 1964. Biological properties and classification of the *Caulobacter* group. Bacteriol. Rev. **28:**231–295.
21. **Quon, K. C., G. T. Marczynski, and L. Shapiro.** 1996. Cell cycle control by an essential bacterial two-component signal transduction protein. Cell **84:**83–93.
22. **Schweizer, H. D.** 1993. Small broad-host-range gentamycin resistance gene cassettes for site-specific insertion and deletion mutagenesis. BioTechniques **15:**831–834.
23. **Sommer, J. M., and A. Newton.** 1989. Turning off flagellum rotation requires the pleiotropic gene *pleD*: *pleA*, *pleC*, and *pleD* define two morphogenic pathways in *Caulobacter crescentus*. J. Bacteriol. **171:**392–401.
24. **Sommer, J. M., and A. Newton.** 1991. Pseudoreversion analysis indicates a direct role of cell division genes in polar morphogenesis and differentiation in *Caulobacter crescentus*. Genetics **129:**623–630.
25. **Volz, K.** 1993. Structural conservation in the CheY superfamily. Biochemistry **32:**11741–11753.
26. **Wang, S. P., P. L. Sharma, P. V. Schoenlein, and B. Ely.** 1993. A histidine protein kinase is involved in polar organelle development in *Caulobacter crescentus*. Proc. Natl. Acad. Sci. USA **90:**630–634.