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# A histidine protein kinase homologue required for regulation of bacterial cell division and differentiation

**(*Caulobacter*/signal transduction/phosphorylation/cell division cycle/*divJ* gene)**

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**ABSTRACT** **Differentiation in the dimorphic bacterium *Caulobacter crescentus* results from a sequence of discontinuous, stage-specific events that leads to the production of a stalked cell and a new motile swarmer cell after each asymmetric cell division. As reported previously, pseudoreversion analysis of mutations in the pleiotropic developmental gene *pleC* identified three cell division genes: *divJ*, *divK*, and *divL*. We show here that one of these genes, *divJ*, encodes a predicted protein of 596 residues with an extensive hydrophobic N-terminal region and a C-terminal domain containing all of the invariant residues found in the family of bacterial histidine protein kinases. Our results also show that *divJ* is discontinuously transcribed early in the swarmer cell cycle during a period that coincides with the $G_1$ to S transition. We propose that the DivJ protein is one member of a signal transduction pathway regulating the cell cycle and differentiation in *Caulobacter* and that protein modification by phosphorylation may play a central role in coupling developmental events to progress through the cell division cycle.**

*Caulobacter crescentus* produces a mother stalked cell and a new swarmer cell at the end of each cell cycle. This invariant pattern of asymmetric cell division provides a useful model for the study of differentiation. Formation of the swarmer cell and its subsequent differentiation into a dividing stalked cell results from a series of discrete morphological events at one pole of the stalked cell, including flagellum assembly, gain of motility, and pilus formation. The new swarmer cell then loses motility, retracts the pili, and sheds the flagellum before differentiating into a stalked cell (reviewed in ref. 1).

A central question in understanding differentiation is the nature of the program that controls the sequence of observed developmental events. Huguenel and Newton (2) have proposed that the temporal and spatial cues required for control of development in *C. crescentus* are furnished by steps in the underlying cell division cycle. Thus, ongoing chromosome replication is required for flagellum biosynthesis (3), and the completion of different steps in the cell division pathway is required for gain of motility and stalk formation (2) and for pilus assembly (4).

Direct genetic evidence that the developmental and cell division cycle pathways are interconnected was provided by a pseudoreversion analysis of temperature-sensitive (ts) mutations in the pleiotropic gene *pleC* (5). Although *pleC* mutants divide normally, they display multiple defects in polar morphogenesis, including the assembly of nonmotile flagella and the failure to form new stalks (6, 7). Mutants blocked early in cell division also assemble inactive flagella at the new cell pole (2), and this similarity in morphologies suggested that *pleC* could be a key gene coupling polar morphogenesis to cell division. Cold-sensitive (cs) suppressors were isolated as motile (mot$^+$) revertants of a ts *pleC* mutant at 37°C and shown to confer a uniformly filamentous phenotype at 24°C (5). These suppressors mapped to three cell division (*div*) genes, *divJ*, *divK*, and *divL*. The *divK* mutation displayed the same cell division and motility phenotypes regardless of the *pleC* allele present, whereas mutations in *divJ* and *divL* displayed a varied pattern of suppression at 24°C and 37°C depending on the *pleC* allele examined.

We have begun to analyze genes identified in this suppressor analysis in order to determine their role in polar morphogenesis and cell division. The first results of these studies, which are reported here, indicate that *divJ* encodes a protein[§] organized into two large domains: an extremely hydrophobic N-terminal region that could anchor the protein in the membrane and a C-terminal region that is similar to the histidine protein kinase domains typical of a large family of bacterial sensor proteins. We also show that *divJ* is periodically transcribed early in the cell cycle at a time that coincides with the $G_1$ to S-period transition. We discuss the possibility that *divJ*, along with *pleC*, *divK*, and *divL*, constitutes one member of an extensive signal transduction pathway involved in cell cycle and developmental regulation in *Caulobacter*.

## MATERIALS AND METHODS

**Bacterial Strains, Plasmids, and Growth.** All *C. crescentus* strains were derived from strain CB15 (ATCC 19089) and were described previously (5, 6) or are described in the text. *Escherichia coli* strains MC1061 and HB101 were used as hosts for cloning and mating, and XL1-Blue (Stratagene) for rescuing phage from pBluescript (Stratagene) clones. *C. crescentus* strains were normally grown at 30°C in PYE or in M2 medium supplemented with antibiotics as described (8). Motility agar contained 0.35% agar in 0.5× PYE (8). Nonpermissive temperatures for cs and ts mutants were 24°C and 37°C, respectively.

**Cloning of the *divJ* gene.** A cosmid pLAFR1-7 (9) library containing random inserts of *C. crescentus* DNA (10) was introduced into PC5388 (*divJ332 zbg350*::Tn*5 str*) by triparental crosses (8) and screened for motile (Mot$^+$) swarming colonies at 37°C in motility agar. The Mot$^+$ Tc$^r$ (tetracycline-resistant) colonies obtained in matings were tested for simultaneous loss of Mot$^+$ and Tc$^r$ to confirm that motility was conferred by the cosmid. Two Tn*5* insertions 70–80% linked

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Abbreviations: ts, temperature-sensitive; cs, cold-sensitive.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M98873).

by transduction to *divJ* were mapped by Southern blot analysis of genomic DNA (9) (see Fig. 2*A*).

**Construction of *lacZ*–Promoter Fusions and Radioimmunoassays of Cells from Synchronous Culture.** DNA fragments a, b, and c (see Fig. 2*B*) were each fused to *lacZ* in the promoter fusion vector pANZ5 (11) to obtain pANZ438, pANZ439, and pANZ436, respectively. β-Galactosidase was assayed according to Miller (12). The rates of synthesis of β-galactosidase and flagellins in a synchronous culture of strain CB15F/pANZ438 were determined by radioimmunoassay (3, 9).

**DNA Sequencing.** Nucleotide sequences of the 2.3-kilobase (kb) fragment containing the *divJ* gene were determined in pBluescript KS(+) and KS(−) by using a series of nested deletions (generated by exonuclease III and mung bean nuclease) and synthetic oligonucleotide primers. The dideoxy chain-termination reaction (13) was carried out with the Sequenase sequencing kit (United States Biochemical).

## RESULTS

**Phenotypes of *pleC* Suppressors Mapping to *divJ*.** The seven cs alleles of *divJ* isolated originally as suppressors of the ts *pleC319* mutation restore motility and bacteriophage φCbK sensitivity at 37°C and confer a cell division defect at 24°C (5). Thus, pseudorevertant strain PC5332 (*divJ332 pleC319*), which displays a clear cs phenotype by replica printing, colony formation, and motility in motility agar, forms a uniform population of long, straight filamentous cells when the culture is shifted from 37°C to 24°C for several generations (Fig. 1*E*). This contrasts with the normal pattern of cell division at 24°C and 37°C observed for both the wild-type strain CB15 (Fig. 1 *A* and *B*) and the parent strain PC5262 containing *pleC319* (Fig. 1 *C* and *D*). Although the suppressors in *divJ* display a less severe division phenotype in a *pleC*$^+$ background, they still fail to divide normally, as shown for *divJ332* (Fig. 1*G*). The *divJ332* allele is unique among the *divJ* suppressors in that it confers a ts motility defect in the wild-type background at 37°C (5).

**Cloning and Complementation Analysis of *divJ*.** Cosmid clone pDIVJ1 was isolated by complementation of the nonmotile phenotype of *divJ332* at 37°C (see *Materials and Methods*) and shown to complement the cs cell division defects of the other previously described *divJ* alleles *divJ334*, *divJ335*, and *divJ336* (ref. 5; data not shown). To locate *divJ* within the insert of clone pDIVJ1, various DNA restriction fragments were subcloned into the broad-host-range plasmid pRK2L1 (14) and used to complement the severe cs cell division defect at 24°C conferred by *pleC319 divJ331* in a *rec*-deficient strain (15) (Fig. 2*A*). Filamentous cells produced by the *divJ* mutants are not motile, and we used the resulting nonmotile phenotype to test for complementation. The *divJ* gene was first localized to the 6.5-kb *Bam*HI–*Bam*HI fragment (pDIVJ15; Fig. 2*A*). Physical mapping of two genetically linked Tn*5* insertions to a *Bam*HI fragment adjacent to the 6.5-kb complementing fragment supported the conclusion that the cloned insert contains the wild-type *divJ* gene and not an outside suppressor (*Materials and Methods*). The complementing fragment was further delimited by subcloning to the 2.3-kb insert in pDIVJ34 (Fig. 2*A*).

**Nucleotide Sequence of *divJ*.** The 2.3-kb DNA fragment in plasmid pDIVJ34 was sequenced on both strands. Analysis of the DNA sequence identified one large open reading frame with a codon usage typical of strain CB15 genes characterized previously. On the basis of this information and the location of the *divJ* promoter (see below; N.O. and A.N., unpublished results), the most probable start codon is the GTG at nucleotide 366, which has a potential upstream ribosome binding site. The reading frame, which terminates at the UGA stop codon at nucleotide 2154 (Fig. 3), would contain 1788 bp and encode a predicted peptide of 596 amino acid residues with a molecular mass of 62,113 Da.

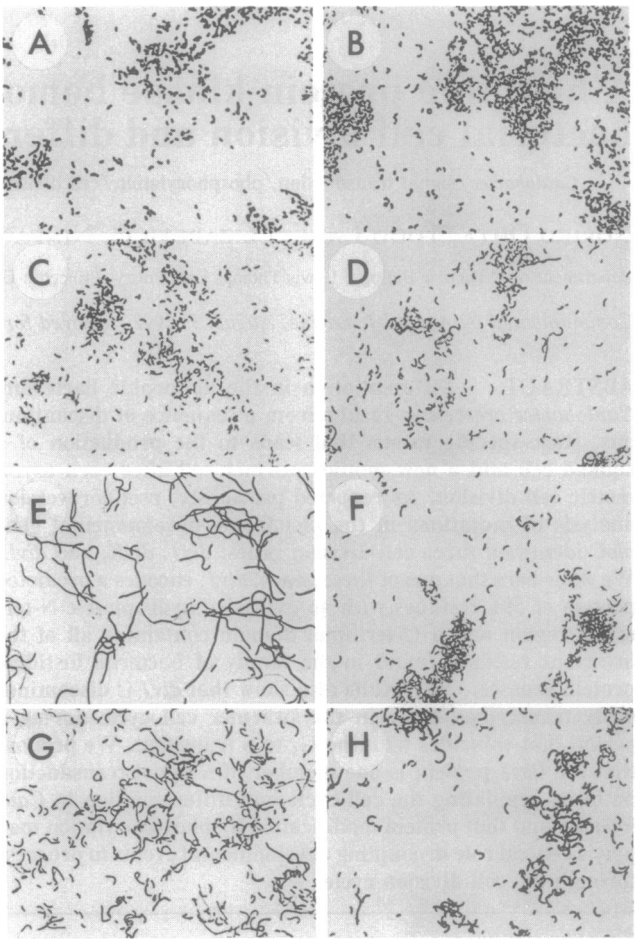


FIG. 1. Phenotypes and morphology of mutants with alleles of cell division gene *divJ* and developmental gene *pleC*. Overnight cultures grown at 37°C in PYE medium were diluted 100-fold and grown to late exponential phase at 24°C and 37°C and examined by light microscopy. (*A* and *B*) CB15 at 24°C and 37°C. (*C* and *D*) PC5262 (*pleC319*) at 24°C and 37°C. (*E* and *F*) PC5332 (*pleC319 divJ332*) at 24°C and 37°C. (*G* and *H*) PC5307 (*divJ332*) at 24°C and 37°C. (×600.)

***divJ* Encodes a Polypeptide with a Histidine Protein Kinase Domain.** Comparison of the DivJ sequence by the method of Lipman and Pearson (16) to the translated sequences in GenBank (Release 70.0) revealed extensive homology between the C terminus of DivJ and the histidine protein kinase domains of several proteins involved in bacterial signal transduction (reviewed in refs. 17 and 18). Residues 320–550 showed ≈39% identity with the putative kinase domain of proteins encoded by the *bvgS* gene in three *Bordetella* species (19, 20). The same region of DivJ showed 33.5% and 22% sequence identity with the kinase domains of the *Bacillus subtilis* PhoR (21) and SpoIIJ (or KinA) (22) proteins, both of which have been shown to have kinase activity. DivJ contains the three conserved regions (I–III) containing invariant residues characteristic of this histidine kinase family, including (I) a histidine at residue 337, which is usually considered to be the site of autophosphorylation; (II) an asparagine at residue 456, which is separated from the histidine by about 100 residues; and (III) the two conserved glycine-rich sequences Asp-Xaa-Gly-Xaa-Gly and Gly-Xaa-Gly, located 20–50 residues C-terminal to the asparagine (Fig. 4).

Determination of the average hydrophobicity of the peptide sequence (23) revealed that the N-terminal region of

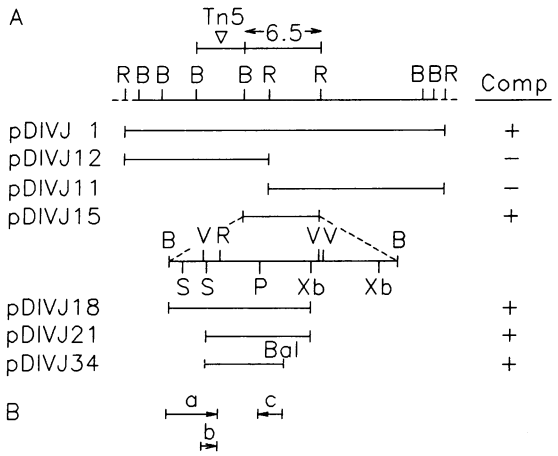


FIG. 2. Location of the *divJ* gene by complementation. (*A*) DNA fragments subcloned in pRK2L1 (14) were examined for complementation of the cs division defect in *rec-526* (15) strain PC5495 (*pleC319 divJ331 rec-526*) in motility agar after stab cultures were incubated at 24°C for 48 hr. +, Large swarms (>5 mm in diameter); −, small swarms (<1 mm in diameter). Complementation (Comp) results were confirmed by the presence of predominantly single, mostly motile, and dividing cells when cells in large swarms were examined by light microscopy. B, *Bam*HI; R, *Eco*RI; V, *Eco*RV; Hc, *Hinc*II; P, *Pst* I; S, *Sst* I; X, *Xba* I; Bal, BAL-31 nuclease deletion endpoint. Broken lines indicate vector sequences. (*B*) DNA fragments fused to *lacZ* were as follows: a, 1.4-kb *Bam*HI–*Eco*RI; b, 0.4-kb *Eco*RV–*Eco*RI; c, 0.4-kb *Pst* I–BAL-31. Arrowheads indicate the endpoints fused to a promoterless *lacZ* gene (11).

≈150 residues is strongly hydrophobic (sequences underlined in Fig. 3) and could contain six membrane-spanning regions. The majority of known histidine kinases are membrane-associated receptor proteins that usually have two membrane-spanning regions in the N terminus with a periplasmic loop which makes up the sensory domain.

The pattern of *pleC* suppression by mutations in *divJ* has suggested that the two genes encode related functions that control polar morphogenesis and cell division in *Caulobacter* (5). Analysis of the translated nucleotide sequence of *pleC* determined by Ely and coworkers predicts that it also encodes a protein typical of the histidine protein kinase family (S. P. Wang, P. Sharma, P. V. Schoenlein, and B. Ely, personal communication) with two potential N-terminal membrane-spanning sequences and a C-terminal kinase domain. DivJ and PleC share 48% amino acid identity over ≈240 residues containing their kinase domains (Fig. 4), and the common functions inferred for the two proteins suggest probable mechanisms for their roles in developmental regulation, as considered below (*Discussion*).

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          319                                                  367
DivJ IDAEALAAGRARFLANMSHELRTPLNAIMGFSDIMRARMFGPLSD.RYAE
PleC KVAESANKAKSEFLANMSHELRTPLNAINGFSEIMMNEMFGPLGDQRYKG
BvgS ESADAANRAKTTFLATMSHEIRTPMNAIIGMLEL...ALLRPADQEPDRQ
PhoR TETKKLEQMRKDFVANVSHELKTPITSIKGFTETLLDGAM..EDKEALSE
KinA MLSEKLS.IAGQLAAGIAHEIRNPLTAIKGFLQ.LMKPTM..EGNE...H
          368                                                  417
DivJ YAELIHESGGHLLDLINDVLDMSKIEAERFELQRGVFDAREAVQAAMRLL
PleC YSQDIHSSGQHLLALINDILDMSKIEAGKMNLKFESMHLEDVAEDAVRLV
BvgS SIQVAYDSARSLLELIGDILDIAKIEAGKFDLAPVRTALRALPEGAIRLF
PhoR FLSIILKESERLQSLVQDLLDLSKIEQQNFTLSIETFEPAKMLGEIETLL
KinA YFDIVFSELSRIELILSELLMLAKPQQNAVK...EYLNLKKLIGEVSALL
          418                                                  466
DivJ RVQSDTAGVQLRGVLPPGEL.EVDADRRALKQIVLNLVSNALKFTPRGGQ
PleC RNRAEAAGLKLDIDFPQLP..EIEADYRAVKQVLLNLLSNAIKFTPRAGS
BvgS DGLARQKGIELVLKTDIVGVDDVLIDPLRMKQVLSNLVGNAIKFTT.EGQ
PhoR KHKADEKGISLHLNVPKDPQY.VSGDPYRLKQVFLNLVNNALTYTPEGGS
KinA ETQANLNGIFIRTSYEKDSIY.INGDQNQLKQVFINLIKNAVESMPDGGT
          467                                                  510
DivJ VTVTA....HGYDGVLEIVVADTGVGISPEDLERLGRPYEQA.GGA.EQR
PleC VTVRVEVRRDPFGDLIKVSVTDTGIGIAKEDLARLAKPFEQV.ESQFSKT
BvgS VVLTVTARPDGEAAHVQFSVSDTGCGISEADQRQLFKPFSQVGGSAEAGP
PhoR VAINVKPREKD....IQIEVADSGIGIQKEEIPRIFERFYRVDKDR.SRN
KinA VDIIITEDEHS....VHVTVKDEGEGIPEKVLNRIGEPFLTTKEK.....
          511                                                  560
DivJ ARGTGLGLSLVRAFAQLLGGEMVIESRLGAGTTVSVRLPVLLAPMVAATP
PleC TQGTGLGLALTKSLITMHDGVLEMHSTPGEGTTVSFTLPVRHSDQKITRD
BvgS APGTGLGLSISRRLVELMGGTLVMRSAPGVGTTVSVDLRLTMIE.KSAQA
PhoR SGGTGLGLAIVKHLIEAHEGKIDVTSELGRGTVFTVTLKRAAEKSA*...
KinA ..GTGLGLMVTFNIIENHQGVIHVDSHPEKGTAFKISFPKK*
```

FIG. 4. Alignment of DivJ sequence with translated nucleotide sequences of genes containing histidine protein kinase domains (arranged in descending order of sequence identity to residues in DivJ; see text for species): PleC (GenBank accession no. M91449); BvgS (19, 20); PhoR (21); KinA, or SpoIIJ (22).

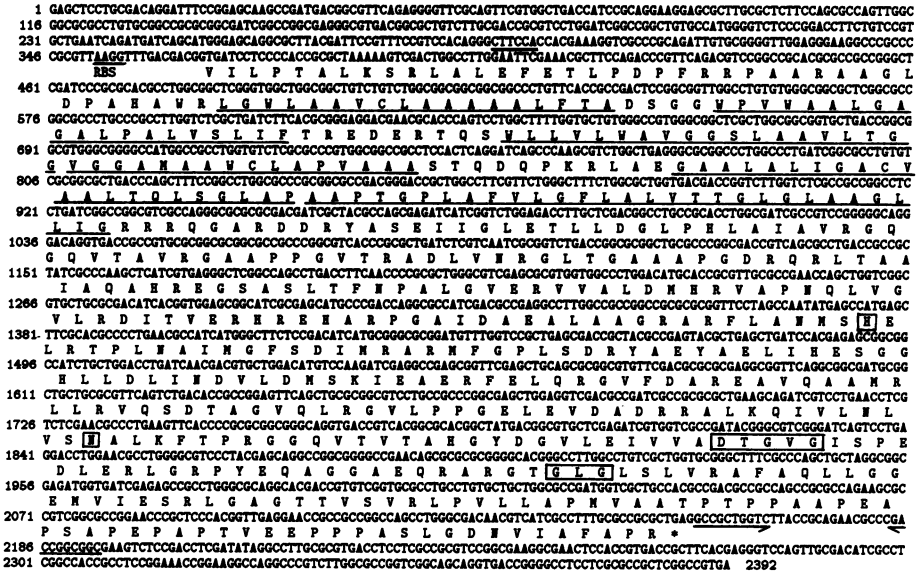

FIG. 3. Nucleotide and translated sequences of *divJ*. The sequence is shown from the *Sst* I site to the endpoint of the BAL-31 deletion. The putative ribosome binding site (RBS) is indicated, and hydrophobic sequences with potential transmembrane regions are underlined. Stem–loop structures for transcription termination are indicated by arrows under the inverted repeats. The *Eco*RI site used for construction of the *lacZ* fusion is overlined. Boxed residues correspond to those discussed in Fig. 4.

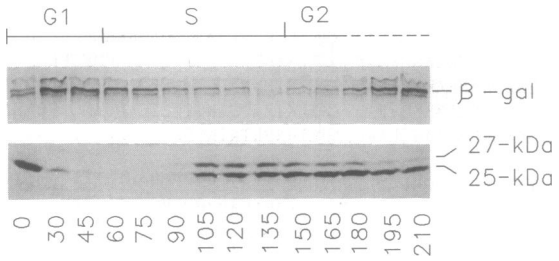


FIG. 5. Cell cycle regulation of *divJ* promoter. (*A*) Synchronous cells of strain CB15F/pANZ438 were pulse-labeled with [$^{35}$S]methionine for 10 min at the times indicated (0–210 min), and the rate of *lacZ* expression driven by the *divJ* promoter was assayed by immunoprecipitation with β-galactosidase antiserum (Promega). The $G_1$, S, and $G_2$ periods of the cell cycle are indicated above the autoradiograms. The second peak of β-galactosidase at 210 min reflects the synthesis in the next cell cycle. The same cell extracts were assayed for the rates of synthesis of 25- and 27-kDa flagellin. β-gal, β-Galactosidase.

**Localization of the *divJ* Promoter and Cell Cycle Regulation.** To locate the *divJ* promoter, DNA fragments a, b, and c (Fig. 2*B*) were fused to the reporter gene *lacZ*, and β-galactosidase activity was assayed. Fusions with fragments a and b gave activities of ≈300 units (12), compared with ≈5 units for the fusion with fragment c and 1–2 units in the wild-type CB15 strain. These results confirm that *divJ* is transcribed from left to right on the map in Fig. 2*A* and show that the *divJ* promoter is located on fragments a and b within the 0.5-kb *Eco*RV–*Eco*RI DNA fragment.

The expression of *divJ* was examined in synchronous cells by determining the rate of β-galactosidase synthesis driven by the *divJ* promoter fused to *lacZ* carried on plasmid pANZ438 in the synchronizable strain CB15F. Our results show that *divJ* is periodically transcribed early in the *Caulobacter* cell cycle, with the maximum rate of expression in mid to late $G_1$ (Fig. 5). The rates of synthesis of 25- and 27-kDa flagellin were determined in the same cell extracts as an internal control (Fig. 5). As expected from previous results (24), the flagellin genes were expressed periodically at the end of the cell cycle, when the two proteins are assembled into the flagellar filament.

## DISCUSSION

A pseudoreversion analysis of the pleiotropic developmental gene *pleC* has implicated at least three *div* genes in the regulation of cell division in *C. crescentus*. The cs suppressors of *pleC* mutations that are defective in the cell division cycle were mapped to *divJ*, *divK*, and *divL* (5). We have described above the molecular cloning, nucleotide sequence analysis, and pattern of cell cycle regulation of one of these genes, *divJ*. These results indicate that *divJ* encodes a protein with an extensive hydrophobic N-terminal region (Fig. 3) and a C-terminal domain containing all of the invariant residues found in a family of bacterial histidine protein kinases (Fig. 4). We have also shown that *divJ* is cell cycle-regulated with a period of transcription that is initiated in the swarmer cell and peaks in mid to late $G_1$, before the initiation of chromosome replication (Fig. 5).

Most of the known histidine protein kinases are members of signal transduction systems that modulate the activity of cognate response regulators by controlling their levels of phosphorylation. The response regulators normally function to activate transcription of target genes, but they also modulate biological responses by other mechanisms, as in the regulation of bacterial chemotaxis by the CheY protein (17, 18). Histidine protein kinases have not been reported to be involved in cell division, and the observation that *divJ* may encode a protein belonging to this family of protein kinases offers a direct biochemical approach to the study of cell cycle regulation and differentiation. It also suggests that protein modification by phosphorylation may play an important role in coordinating these two important developmental processes.

The recent finding that *pleC*, like *divJ*, encodes a protein with a highly conserved histidine protein kinase domain (S. P. Wang, P. Sharma, P. V. Schoenlein, and B. Ely, personal communication) strongly suggests that the cs suppressor mutations in *divJ* compensate for *pleC* mutant defects by altering phosphorylation of a response regulator(s). Consistent with this interpretation is our earlier observation that the degree of suppression by a given *divJ* or *divL* mutation depended on the *pleC* allele examined (5). One candidate for a response regulator gene is *divK*. Mutations in this gene act as bypass suppressors of all *pleC* alleles (5), and the translated nucleic acid sequence of *divK* contains the structural elements conserved in the N-terminal domains of bacterial response regulators (G. Hecht and A.N., unpublished data).

One model for the interactions among the genes discussed above is shown in Fig. 6. Here we assume that DivJ and PleC are membrane-associated protein kinases, which along with DivK and perhaps other, unidentified gene products are members of a complex signal transduction pathway that regulates both cell division and polar morphogenesis (gain of motility and stalk formation). Although the signal activating DivJ and PleC is not known, we propose that either of the two kinases can directly or indirectly alter the level of phosphorylation of an acceptor protein such as DivK. DivK or another phosphorylated intermediate could then act directly to affect developmental regulation or, alternatively, be part of a multicomponent "phosphorelay" like the one active early in *B. subtilis* sporulation that couples kinases KinA and KinB to the response regulator Spo0A (25).

The model as outlined is reminiscent of the *E. coli* signal transduction system in which the two sensor kinases ArcB and CpxA act through the same response regulator (ArcA) to control anaerobic metabolism and pilus formation, respectively (discussed in ref. 26). In a similar fashion, the division defects in *Caulobacter* are most closely associated with mutations in *divJ*, and defects in motility and stalk formation with mutations in *pleC*. Fig. 6 is only a working model, and it is evident that the results obtained thus far could also be explained if DivJ and PleC had different cognate response regulators and the observed suppression resulted from modification of one response regulator by the other regulatory system—e.g., cross regulation as observed in the phosphate (or PHO) system (27). The potential complexity of these interactions is underlined by the facts that the division phenotype of *divJ* mutations is potentiated by the presence of certain *pleC* alleles (see Fig. 1) and that the function of *divL*,

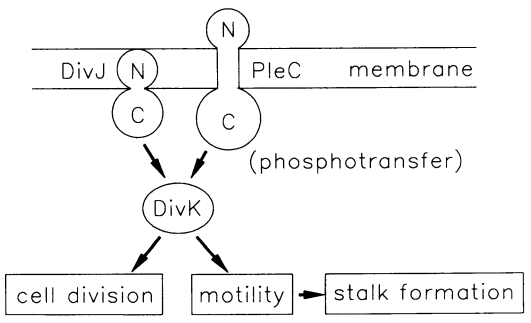


FIG. 6. Model for roles of DivJ, DivK, and PleC in cell division and polar morphogenesis. N- and C-terminal regions of DivJ and PleC are indicated.

which appears from genetic analysis to play a role comparable to that of *divJ* (5), has not been assigned.

The periodic transcription of *divJ* (Fig. 5) raises the general question of whether temporal regulation is a general feature of bacterial cell cycle genes and essential to their function. We have previously shown that *dnaC*, a *Caulobacter* gene required for DNA chain elongation, is also maximally transcribed during a restricted period just before the initiation of chromosome replication (9). Pseudorevertant strains containing *divJ* alleles form uniform populations of long, straight filamentous cells at 24°C (Fig. 1*E*). This result, which suggests that *divJ* may act early in division, is consistent with the timing of its expression. The *pleC* gene product is known to be required later in the cell cycle, from mid-S period until the cell gains motility just before division (6), but the regulation of its expression has not been examined.

In summary, our results show that the *C. crescentus* cell division gene *divJ* encodes a putative histidine protein kinase that is periodically transcribed early in the cell cycle. We suggest that the products of the *divJ*, *divK*, and *pleC* genes are members of a signal transduction pathway regulating the cell cycle and differentiation and that protein modification by phosphorylation may play a central role in coupling developmental events to progress through the cell division cycle.

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