Pseudoreversion Analysis Indicates a Direct Role of Cell Division Genes in Polar Morphogenesis and Differentiation in *Caulobacter crescentus*

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ABSTRACT

A pseudoreversion analysis was used to examine the role of cell division genes in polar morphogenesis in *Caulobacter crescentus*. Extragenic suppressors of temperature sensitive mutations in *pleC*, a pleiotropic gene required for cell motility, formation of polar φCbK bacteriophage receptors, and stalk formation, were isolated. These suppressors, which restored motility at 37°, simultaneously conferred a cold sensitive cell division phenotype and they were mapped to the three new cell division genes *divJ*, *divL* and *divK*. The cold-sensitive mutations in *divL*, and to a lesser extent *divJ*, exhibited a relatively narrow range of suppression. The cold-sensitive cell division mutation in *divK*, by contrast, suppressed all *pleC* mutations examined and behaved as a classical bypass suppressor. The direct role of this cell division gene in the regulation of motility is suggested by the observation that *divK341* mapped to the same locus as *pleD301*, a pleiotropic mutation that prevents loss of motility and stalk formation. These results provide strong evidence that the cell division and developmental pathways are interconnected and they support our earlier conclusion that cell division is required for the regulation of polar morphogenesis and differentiation in *C. crescentus*.

ASYMMETRIC cell division is a central mechanism for the formation of differentiated cell types, and the pattern of division in *Caulobacter crescentus* has provided a useful model system for studying the mechanisms regulating this developmental process (SHAPIRO 1985; NEWTON 1989; NEWTON and OHTA 1990). These aquatic bacteria divide unequally to produce two different cells, the nonmotile stalked cell and a new, motile swarmer cell with a single polar flagellum. Formation of the swarmer cell and its differentiation into a stalked cell result from a series of discrete morphological events at the stalk-distal pole of the dividing cell (see cell cycle in Figure 1). The first of these is flagellum assembly, which depends on over 50 flagellin (*fla*) genes (NEWTON and OHTA 1990; NEWTON 1989). A subsequent sequence of events includes activation of flagellum rotation (gain of motility), biosynthesis of polar bacteriophage receptors, pili formation, loss of motility, and stalk formation (SOMMER and NEWTON 1988). A pleiotropic gene required for these latter events, *pleC*, is the subject of the pseudoreversion analysis described in this paper.

We have proposed that the temporal and spatial control of developmental events in *C. crescentus* is ultimately regulated by steps in the cell division cycle (HUGUENEL and NEWTON 1982). The requirement of chromosome replication for flagellum formation appears to be exerted at the level of flagellar (*fla*) gene expression (SHEFFREY and NEWTON 1981) and the analysis of cell division mutants has suggested that completion of early cell division steps are required for gain of motility and stalk formation at the flagellated cell pole (HUGUENEL and NEWTON 1982; see Figure 1). More recent studies have also indicated a requirement of cell separation, the last step in cell division, for polar morphogenesis: cells blocked at this step gain motility normally, but they fail to assemble pili (SOMMER and NEWTON 1988).

To determine if the requirement of cell division for polar morphogenesis reflects the direct involvement of cell division genes, we have undertaken a pseudoreversion analysis of pleiotropic, developmental mutations in *pleC*. The similarities in phenotypes between *pleC* mutants that assemble nonmotile flagella and do not form stalks (SOMMER and NEWTON 1989; FUKUDA, IBA and OKADA 1977; Figure 1B) and the polar morphology of some filamentous cell division mutants that also assemble nonmotile flagella and do not form stalks at the new cell pole (HUGUENEL and NEWTON 1982; Figure 1C) suggested to us that *pleC* might play a key role in coupling polar morphogenesis to cell division. Motile revertants of temperature sensitive (Ts) *pleC* mutants were isolated at the nonpermissive temperature of 37° and then screened for extragenic suppressors that simultaneously conferred a cold-sensitive (Cs) cell division phenotype at 24°. The assumption in this approach was that a deleterious mutation in *pleC* can be compensated for by mutations in other genes that encode proteins that normally affect *pleC*

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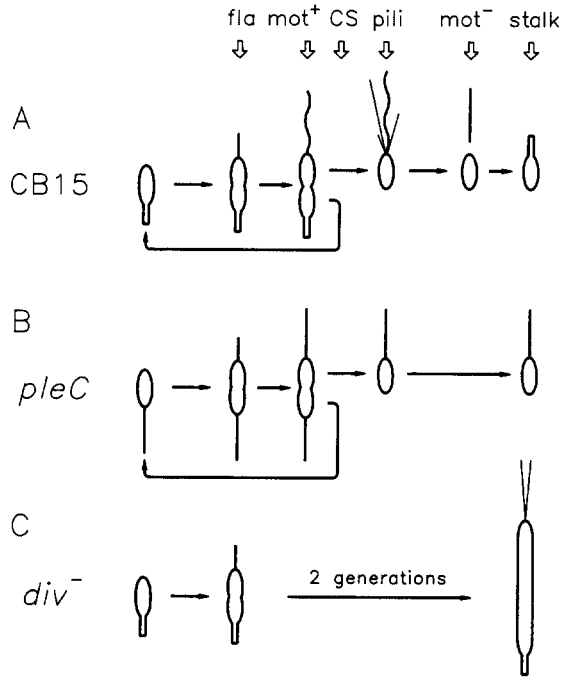

FIGURE 1.—*C. crescentus* cell cycle. A, The sequence of developmental events in the wild type strain CB15 includes flagellum formation (fla), activation of flagellum rotation (mot$^+$), cell separation (CS), pili formation (pili), loss of motility (mot$^-$), and stalk formation. B, *pleC* mutants assemble inactive flagella (designated as straight lines), they are mot$^-$, bacteriophage φCbK resistant and fail to form a stalk, but they divide normally. C, Cells blocked early in cell division (*div*$^-$) also assemble inactive flagella and do not form a stalk at the cell pole.

function. It should be possible to identify such mutations as extragenic suppressors.

Pseudoreversion studies have been extremely useful in the study of how genes function in complex regulatory pathways. This approach was used to identify interacting gene products in bacteriophage P22 assembly (JARVICK and BOTSTEIN 1975), to analyze the yeast cell cycle (MOIR and BOTSTEIN 1982) and cytoskeleton (NOVICK, OSMOND and BOTSTEIN 1989), and to study the allele-specific interaction of *che* and *fla* genes in *Escherichia coli* (PARKINSON *et al.* 1983) and in *Salmonella typhimurium* (YAMAGUCHI *et al.* 1986). In previous pseudoreversion studies revertants have been examined at the second temperature for a phenotype similar to that conferred by the original mutation. Our analysis differs in that revertants of the original nonmotile strain were examined for the acquisition of a new phenotype, namely a defect in an essential cell division function. This procedure allowed us to identify Cs mutations in three previously unidentified cell division genes, *divJ*, *divK*, and *divL*. Our results indicate that the cell division and developmental pathways are interconnected and they support a model for developmental regulation of *C. crescentus* in which cell division genes play a direct role in polar morphogenesis and cell differentiation (HUGUENEL and NEWTON 1982).

MATERIALS AND METHODS

Strains and growth conditions: Wild-type *C. crescentus* strain CB15 (American Type Culture Collection 19089) and motility and cell division mutants were routinely grown at 30° in peptone-yeast extract (PYE; POINDEXTER 1964) medium. All *pleC* point mutations used in this study were isolated after UV-light irradiation (SOMMER and NEWTON 1989). These strains, as well as the *divJ*, *divK*, and *divL* mutants, are listed in Table 1.

Isolation of cold-sensitive suppressor mutations: Strain PC5262 carrying the Ts *pleC319* allele (SOMMER and NEWTON 1989) was inoculated into motility agar [½ PYE + 0.35% agar (Difco)] and grown at 37°. Cells picked from individual motile flares produced from spontaneous revertants were patched onto PYE plates, grown overnight at 37°, and replica printed at 24°. Colonies that grew poorly at 24° were purified at 37° and analyzed by phase contrast microscopy for defects in cell division after an extended period of growth at 24°. Each of the original motile revertants was also screened for Cs motility defects.

Genetic mapping: The *pleC319* allele in each Cs revertant was replaced with the *ple*$^+$ allele by transduction with a bacteriophage φCr30 lysate (ELY, CROFT and GERARDOT 1984) made on Tn*5* insertion mutant PC5347. This strain contains *zhf-340*::Tn*5* which is 30% linked to the wild type *pleC* locus. Twenty to 40 kanamycin resistant (Km^r) recombinants were then screened for Cs defects in cell division and motility; presence of the *pleC*$^+$ allele was verified by back crossing the linked Tn*5* insertion into strain CB15. With the exception of strain PC5318 (*pleC319*, *divK341*), presumptive *pleC*$^+$ recombinants were recovered from all crosses that displayed altered cell division or motility phenotypes (see Figure 2 below) at a frequency expected for replacement of the *pleC319* allele by *pleC*$^+$. Tn*5* insertions linked to the Cs suppressor mutations were isolated using a pool of bacteriophage φCr30 lysates made on strain CB15 cells containing random Tn*5* insertions (orgy lysate), as described previously (OHTA, MASUREKAR and NEWTON 1990). Recombinants of the original Cs revertants that had been transduced with the lysate were selected for Km^r and screened for loss of the cell division defect (SOMMER and NEWTON 1989). Recombinants containing the Tn*5* insertion linked to the wild-type allele of the Cs suppressor were verified by back crossing the Tn*5* insertion into the original Cs revertant strain and screening Km^r recombinants for loss of the Cs division phenotype. The Tn*5* insertion in a cold sensitive recombinant was then used to move the suppressors allele into the wild-type background. These crosses are illustrated in Table 1 by the construction of strains PC5343 and PC5432 which contain insertion element *zzz354*::Tn*5* linked to *divJ332*.

Phenotype assays: Motility was assayed by swarm formation in motility agar. Cs recombinants were usually analyzed by phase contrast microscopy to distinguish between nonmotile strains and cell division mutants, both of which produce tight colonies on motility agar. Cell morphology was always assayed by phase contrast microscopy. Stalk formation was determined by phase contrast microscopy on a smear of cells immediately after drying at 500× magnification without a coverslip and confirmed in some instances by electron microscopy. Bacteriophage φCbK resistance was assayed by suspending parts of a colony in a loop of PYE broth and streaking it across the phage on a fresh PYE plate.

RESULTS

Cold-sensitive suppressors of *pleC*: We isolated 350 spontaneous motile revertants of the Ts *pleC*

TABLE 1

Bacterial strains

Strain	Genotype	Source or construction[a]
CB15	Wild type	ATCC 19089
PC5225	*pleC301*::Tn*5*	SOMMER and NEWTON (1989)
PC5253	*pleC358*::Tn*5*	This work
PC5255	*pleC314*	SOMMER and NEWTON (1989)
PC5258	*pleC315*	This work
PC5262	*pleC319*	SOMMER and NEWTON (1989)
PC5264	*pleC321*	SOMMER and NEWTON (1989)
PC5267	*pleC322*	This work
PC5270	*pleC323*	SOMMER and NEWTON (1989)
PC5281	*pleC330*	SOMMER and NEWTON (1989)
PC5282	*pleC331*	This work
PC5283	*pleC343*	This work
PC5287	*pleC335*	This work
PC5302	*pleC319, pleD301, zzz-351*::Tn*5*	Orgy lysate × PC5316 → Km^r[Div$^+$]
PC5309	*pleC358*::Tn*5, pleD301, divJ332*	ϕ(PC5253) × PC5316 → Km^r[Ple$^-$]
PC5310	*pleC358*::Tn*5, pleD301*	ϕ(PC5309) × CB15 → Km^r[mot$^+$]
PC5315	*pleC319, divJ331*	This work
PC5316	*pleC319, pleD301, divJ332*	SOMMER and NEWTON (1989)
PC5317	*pleC319, divJ333*	This work
PC5318	*pleC319, divK341*	This work
PC5319	*pleC319, divL342*	This work
PC5320	*pleC319, divL343*	This work
PC5321	*pleC319, divJ334*	This work
PC5323	*pleC319, divJ335*	This work
PC5325	*pleC319, divJ336*	This work
PC5326	*pleC319, divJ337*	This work
PC5327	*pleC319, divJ338*	This work
PC5343	*pleC319, pleD301, zzz-354*::Tn*5, divJ332*	ϕ(PC5344) × PC5316 → Km^r[Div$^-$]
PC5344	*pleC319, pleD301, zzz-354*::Tn*5*	Orgy lysate × PC5316 → Km^r[Div$^+$]
PC5345	*pleC319, pleD301, divJ332, zhf341*::Tn*5*	SOMMER and NEWTON (1989)
PC5346	*pleC319, pleD301, divJ332, hunE112*::Tn*5*	SOMMER and NEWTON (1989)
PC5347	*zhf-340*::Tn*5*	Orgy lysate × PC5262 → Km^r[Ple$^+$]
PC5353	*divJ332, hunE112*::Tn*5*	ϕ(SC1588) × PC5316 → Km^r[Ple$^+$]
PC5385	*pleC319, zzz-353*::Tn*5*	Orgy lysate × PC5319 → Km^r[Div$^+$]
PC5386	*pleC319, divL342, zzz-353*::Tn*5*	ϕ(PC5385) × PC5319 → Km^r[Div^{cs}]
PC5432	*zzz354*::Tn*5, divJ332*	ϕ(PC5343) × CB15 → Km^r[Div$^-$]
SC1588	*hunE112*::Tn*5, str152*	ELY, CROFT and GERARDOT (1984)

[a] Allele *divJ332* is referred to as *divJ302* in SOMMER and NEWTON (1989). Abbreviations: Km^r, resistance to kanamycin; mot$^+$, motile; ple$^-$, ϕCbK resistant and mot$^-$; ple$^+$, ϕCbK sensitive and mot$^+$; Div, defective in cell division.

strain PC5262 at 37°. The revertants were then screened for second site mutations that suppressed the nonmotile (mot$^-$) phenotype of the *pleC319* allele at 37° and simultaneously conferred a Cs phenotype at 24° (MATERIALS AND METHODS). Eleven Cs revertants were identified and all of them produced long straight, largely unpinched filaments after prolonged incubation at 24° (Table 1; strains PC5315–PC5327). A similar frequency of Cs strains was found among the revertants of Ts strain PC5255 (*pleC314* SOMMER and NEWTON 1989). None of the 500 motile revertants of the *pleC314* and *pleC319* mutants isolated at 37° was found to be Cs for motility alone.

We examined the phenotypes of revertant strains that contained a suppressor mutation in each of the three linkage groups defined below (*divJ*, *divK* and *divL*) in greater detail at the nonpermissive temperature. The rate of DNA synthesis in these strains continued at the wild type rate after shifting the cells from 37° to 24° (data not shown), as measured by the incorporation of [^{3}H]guanosine (OSLEY and NEWTON 1980). Although the Cs mutants did not form colonies at 24°, the cells continued to grow at this temperature for several generations as measured by the increase of optical density. The cell division defects were rapidly reversed when the cells were shifted back to 37°. Based on this analysis (OSLEY and NEWTON 1980), we concluded that the primary effect of the suppressors is on cell division.

Mapping suppressor mutations to *divJ*, *divK* and *divL*: The *pleC319* allele was replaced by the *pleC*$^+$ allele in the 11 Cs revertants using a Tn*5* insertion element tightly linked to the *pleC* locus (MATERIALS AND METHODS). All Km^r recombinants isolated from 10 of the strains retained at least partial cold sensitivity for cell division, indicating that the suppressors are

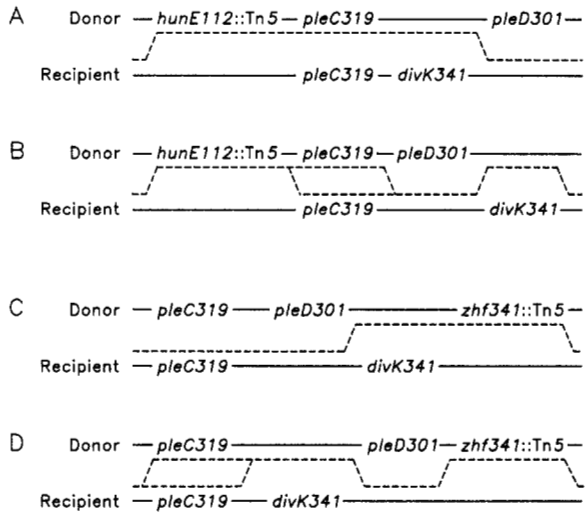

FIGURE 2.—Mapping of *divK341* to the *pleD* locus. The dashed lines indicate the crossover events required to produce nonmotile kanamycin resistant recombinants, depending on the relative order of *pleD* and *divK*. A total of 160 kanamycin-resistant recombinants were scored from each of these two crosses for motility. An additional 160 kanamycin-resistant recombinants from a similar cross in which a Tn*5* insertion in *pleC* (PC5310, *pleC358*::Tn*5*, *pleD301*) was used as the selectable marker when transduced into strain PC5318 were screened for motility. All 480 recombinants were found to be motile at 37°, indicating recombination had not occurred between the *pleD301* and *divK341* alleles.

extragenic and unlinked to *pleC*. The suppressor (*divK341*) in one revertant (PC5318) was also extragenic, but linked to *pleC* (see below). Tn*5* insertions linked by transduction to each of the Cs suppressors were isolated (MATERIALS AND METHODS) and used in phage ϕCr30 crosses to place these cell division genes in three linkage groups. No genetic linkage between the genes identified here and previously published cell division genes (OSLEY and NEWTON 1980) was observed.

divK: The Cs suppressor in strain PC5318, which is 50% linked to *pleC301*::Tn*5* (PC5225), was designated as *divK341* (see Figure 2). This linkage is approximately the same as that observed between *pleC* and *pleD301*, a mutation reported to act as a bypass suppressor of the *pleC* motility defect (SOMMER and NEWTON 1989), but not to confer a cell division phenotype. The *pleD* mutant, which is blocked at a step required for loss of motility, retains motility throughout the cell cycle and does not form stalks (SOMMER and NEWTON 1989). We originally attempted to order the two suppressors genetically using three factor crosses. The donor strain contained the *pleC319* and *pleD301* mutations along with either the Tn*5* insertion *hunE112*::Tn*5* (Figure 2, A and B) or *zhf-341*::Tn*5* (Figure 2, C and D) which are located on either side of the *pleC/pleD* gene cluster (SOMMER and NEWTON 1989) and used as the selected markers. The recipient strain PC5318 also contained *pleC319* along with *divK341*. At 37° both *pleD301* and *divK341* suppress the nonmotile phenotype of *pleC319*. Because *pleC319* was present in both the donor and recipient strain, nonmotile *pleD*$^+$, *divK*$^+$ recombinants would be expected if recombination occurred between the *divK341* and *pleD301* alleles. In none of the 480 recombinants examined from these and similar crosses (see Figure 2 legend), however, did we recover a nonmotile strain (*pleC319*, *pleD*$^+$, *divK*$^+$). The failure to observe recombination between *divK341* and *pleD301* suggests that the two mutations are very closely linked and possibly allelic.

divL: A second linkage group was identified using insertion *zzz-353*::Tn*5* (PC5385), which is approximately 30% linked to the Cs suppressors in strains PC5319 and PC5320. The two revertant alleles were designated *divL342* and *divL343*, respectively. The *divJ* and *divL* loci are unlinked by transduction.

divJ: *zzz-351*::Tn*5* (PC5302) was shown to be 40% linked to suppressor *divJ332* in strain PC5316 and the linked Tn*5* insertion used to transduce the *divJ*$^+$ allele into the other *pleC* revertants. Analysis of the recombinants showed that Cs suppressors in seven strains (PC5315 [*divJ331*], PC5317 [*divJ333*], PC5321 [*divJ334*], PC5323 [*divJ335*], PC5325 [*divJ336*], PC5326 [*divJ337*] and PC5327 [*divJ338*]; Table 1) were also approximately 40% linked to *zzz-351*::Tn*5*, which allowed us to assign them to the *divJ* locus. The recent isolation of a cloned *C. crescentus* DNA fragment which complements all of these mutations further confirms that the suppressors map to the same locus (E. NINFA, J. M. SOMMER and A. NEWTON, unpublished).

Characterization of the cold-sensitive suppressors: The Tn*5* insertions linked to *divJ* (*zzz-351*::Tn*5* or *zzz-354*::Tn*5*), *divL* (*zzz-353*::Tn*5*) and *divK* (*zhf-341*::Tn*5*) were used to back-cross the suppressor mutations into strain CB15 to assess the phenotypes of the suppressor in the wild type genetic background (Figure 3, first row) and into the *pleC319* mutant background to confirm the mapping and observed phenotypes of the original revertant strains (Figure 3, second row). Recombinant strains were examined both at 37° and 24° for motility and cell division and scored as described in the legend to Figure 3. Open boxes indicate essentially wild-type motility or cell division, filled boxes a defective motility or cell division phenotype, and shaded boxes an intermediate defect.

All of the suppressors displayed the same motility and cell division phenotypes when they were back crossed into the *pleC319* genetic background that we had observed in the original pseudorevertants (Figure 3, second row), which argues that each suppressor resulted from a single mutation. When the suppressors were crossed into the *pleC*$^+$ background (Figure 3, first row) the *divL* and *divK* alleles displayed a phe-

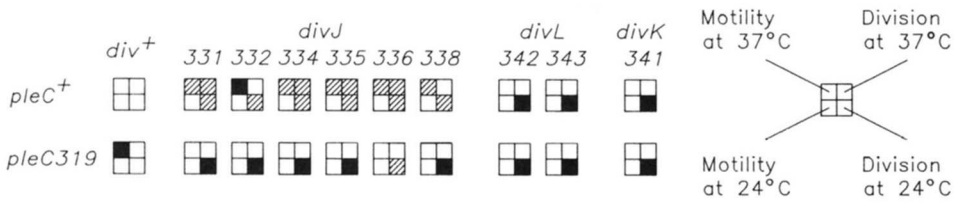

FIGURE 3.—Motility and cell division phenotypes of *divJ*, *divK* and *divL* mutations. Tn*5* insertions linked to the *divJ*, *divK* and *divL* alleles were used to transduce the suppressors into strains CB15 and PC5262 (*pleC319*). Motility and cell division morphology were scored by light microscopy of cells taken from motility agar. Open boxes indicate wild type motility or cell division (more than 50% normal motility or less than 5% filamenting cells), filled boxes indicate cells defective in motility or cell division (uniformly nonmotile cells or very long filamentous cells), and shaded boxes represent an intermediate defect (a mixture of partially motile single cells and filaments of up to 5 cell lengths). The cell division phenotype could also be scored by the inability of defective mutants (closed boxes) or impaired ability of partially defective mutants to form colonies at the nonpermissive temperature (shaded boxes). Filamentous cells displayed no motility at 24° or 37° and in cultures containing mixed filaments and single cells, motility was always scored on single cells.

notype identical to that observed in the *pleC319* background and the *divJ* mutations conferred a partial defect in cell division at 24°. Thus, the Cs cell division phenotype is a characteristic of the extragenic suppressors; only in the case of the *divJ* mutations was the phenotype more extreme in the presence of the mutant *pleC* allele. All of the *divJ* alleles conferred substantially less motility at 37° in the *pleC*$^+$ background than in the *pleC319* background, and most strikingly, *divJ332* was completely nonmotile at 37° in the wild-type background.

Pattern of *pleC* suppression by *divK*, *divL* and *divJ* alleles: The specificity of the suppressors was examined in more detail by transducing them into strains with different *pleC* point mutations (SOMMER and NEWTON 1989) using the linked Tn*5* insertions (Figure 4). Km^r recombinants were selected at 37° and the double mutants were scored for motility and cell division at 24° and 37° as described in Figure 3. Suppression of *pleC301*:: Tn*5* was tested by transducing the Tn*5* into the original Cs *pleC319* revertants by selecting for Km^r.

divK341: The *divK341* allele was introduced into the *pleC* mutant backgrounds by selection for the Km^r marker of the *zhf-341*::Tn*5* insertion that is 44% linked to the *divK* and 30% linked to *pleC* (SOMMER and NEWTON 1989). Because the *pleC* marker would be lost in about one-third of the crosses, we analyzed at least 20 Km^r recombinants for each *pleC* mutant tested. A total of 29 different *pleC* mutations, including the 11 shown in Figure 4, were examined. *divK341* completely suppressed the motility defect of all *pleC* mutations examined, including the Tn*5* insertion *pleC301*::Tn*5*, and the patterns of motility and cell division in all of the mutant *pleC* backgrounds (Figure 4) were identical to that observed originally in *pleC319* (Figure 3). These results led us to conclude that *divK341* acts as a bypass suppressor of *pleC* mutations. The suppression of *pleC301*::Tn*5* also suggested that *pleC* is not essential for motility.

divL: Suppressors *divL342* and *divL343* displayed a much narrower range of specificity than the *divK* allele. Other than the original mutation *pleC319*, *divL343* fully suppressed the motility defect of only 2 of the 6 *pleC* point mutations examined at 37°, while *divL342* fully suppressed the motility of 5 of the 11 point mutations. When both motility and cell division were examined at 24° and 37° the patterns of suppression were quite diverse, particularly with *divL343*: each of the *pleC* point mutations examined produced a different suppression pattern (Figure 4). Although this type of result is normally associated with the behavior of interaction suppressors, the *divL342* and *divL343* alleles also suppressed the motility defect of *pleC301*::Tn*5* (Figure 4), which is discussed below (Discussion).

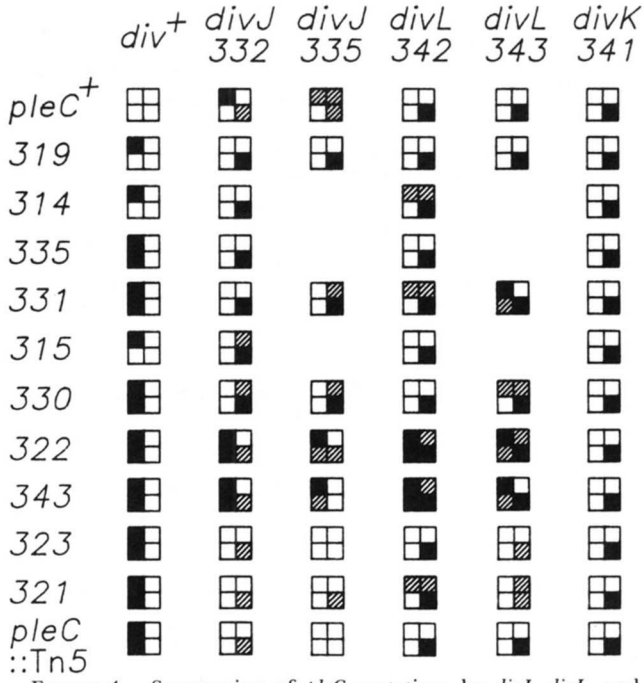

FIGURE 4.—Suppression of *pleC* mutations by *divJ*, *divL*, and *divK* alleles. *divJ332*, *divJ335*, *divL342*, *divL343* and *divK341* were transduced into different *pleC* point mutants and *pleC301*::Tn*5* was transduced into the suppressor strains as described in the text. The resulting double mutants were analyzed for motility and cell division as described in Figure 3.

divJ: *divJ332* and *divJ335* displayed a broader range of suppression than the *divL* alleles in that they re-

stored motility at 37° to most of the *pleC* mutations examined, including the Tn*5* insertion mutation *pleC301*::Tn*5* (Figure 4). None of the *divJ* mutations examined, including those shown in Figure 3, suppressed *pleC322* and *pleC343* (Figure 4), however. Also, the 6 *divJ* mutant alleles displayed less motility at 37° in the *pleC*$^+$ than in the original *pleC319* background (Figure 3). Thus, the mechanism of suppression by the *divJ* mutations is not clear from these results (see DISCUSSION).

The two *pleC* mutations (*pleC322* and *pleC343*) that were not suppressed for motility at 37° by the Cs *divJ* and *divL* alleles (Figure 4) were shown to revert to *mot*$^+$ at approximately the same frequency as other *pleC* mutants (data not shown). This result indicates that the mutations are not deletions and that the *pleC322* and *pleC343* strains used in these experiments do not contain additional motility mutations that would have interfered with the suppression tests. Furthermore, in a more recent pseudoreversion study of *pleC* we have isolated Ts mutations in *divL* that completely suppress the *mot* defects of *pleC322* and *pleC343* at 24° (G. HECHT and A. NEWTON, unpublished).

Revertants of *divJ* map to *pleC*: The behavior of the *divJ* locus was further investigated by isolating suppressors of *divJ332*. To do this we took advantage of the nonmotile phenotype conferred by *divJ332* at 37° in a *pleC*$^+$ background (Figure 3). Independent *mot*$^+$ revertants were isolated at the high temperature from strain PC5353, which contains *divJ332* and a Tn*5* insertion (*hunE112*::Tn*5*) closely linked to the *pleC* locus (SOMMER and NEWTON 1989). Bacteriophage φCr30 lysates were prepared on each revertant, the *pleC* region transduced into strain CB15 by selecting for Km^r, and 10 recombinants scored for motility and phage resistance at 37° and 24°. Eight of the 48 *mot*$^+$ revertants of strain *divJ332* were found to be outside suppressors and to map to *pleC* as judged by their close linkage to *hunE112*::Tn*5*, their nonmotile, stalkless phenotype, and their resistance to phage φCbK (data not shown); this conclusion can be confirmed by complementation of the suppressor alleles with a *pleC*$^+$ clone. The isolation of suppressors of *divJ332* that map back to *pleC* argues at a minimum that the *divJ* mutations analyzed here do not act as informational suppressors.

DISCUSSION

Pseudoreversion analysis has been applied previously in genetic studies of yeast, bacteria, and bacteriophage development (JARVICK and BOTSTEIN 1975; MOIR and BOTSTEIN 1982; NOVICK, OSMOND and BOTSTEIN 1989; PARKINSON *et al.* 1983; YAMAGUCHI *et al.* 1986). We have extended and modified this approach to the study of developmental regulation in *C. crescentus*. Starting with a Ts mutation in *pleC*, a gene required for motility, polar phage receptor site formation and stalk formation, we have isolated suppressors that restored the mot$^+$ phenotype of a *pleC* mutation at 37° and simultaneously conferred a Cs defect in cell division. Genetic analysis of eleven such suppressors showed that they retained a partial Cs phenotype in the absence of the *pleC* mutations and mapped to three previously unidentified genes, *divJ*, *divK* and *divL*. Thus, the selection of conditional pseudorevertants of developmental mutants of *C. crescentus* is a fruitful approach to the identification of new cell division genes.

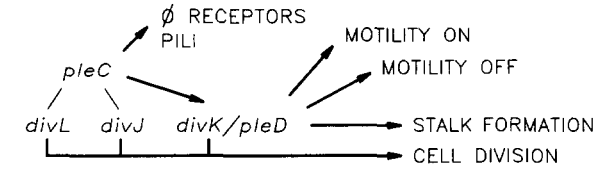

FIGURE 5.—Working model for organization and function of cell division genes in the *pleC/pleD*-dependent pathway. The proposed functional relationship of the genes is based on the analysis of *pleC* suppression by the *pleD*, *divJ*, *divK* and *divL* mutations described in the text (see DISCUSSION). The pattern of pili assembly generally paralleled that shown for phage sensitivity (data not shown). These results suggest that the developmental pathway branches to phage receptor and pili formation at the *divJ/pleC/divL* step(s) and to flagellum activation at or after *divK/pleD* step.

We have reported in an earlier study that steps in the DNA synthetic (SHEFFREY and NEWTON 1981) and cell division (HUGUENEL and NEWTON 1982; SOMMER and NEWTON 1988) pathways are required for flagellum formation, motility and spatial localization of polar structures, and on the basis of these results we have proposed that development in *C. crescentus* is coupled to steps in the cell division cycle (HUGUENEL and NEWTON 1982). The isolation of Cs cell division mutations as suppressors of pleiotropic developmental mutations in *pleC* now provides direct evidence for this conclusion; the results indicate that the cell division and developmental pathways are interconnected and the different behaviors of the suppressors mapping to *divK* on the one hand and to *divJ* and *divL* on the other suggest that more than a single mechanism may be involved.

Results obtained with the *divK* allele are the easiest to interpret. In contrast to other suppressors, *divK341* suppressed all *pleC* mutations tested and it behaved like a classical bypass suppressor at 37° (Figure 4). These findings suggest that cell division gene *divK* acts after *pleC* in the pathway regulating cell motility and stalk formation, as diagrammed in Figure 5. *pleD301*, like *divK341*, acts as a bypass suppressor of the *mot* phenotype of *pleC* mutations, but it does not confer a cell division defect (SOMMER and NEWTON 1989). Another difference between the two suppressors is that strains containing *divK341* show a normal, stage-specific pattern of motility and stalk formation

in the cell cycle at 37°, while strains containing *pleD301* fail to turn off cell motility, do not form stalks, and are motile throughout the entire cell cycle (SOMMER and NEWTON 1989). *divK341* and *pleD301* could not be separated by recombination (Figure 2) and if the two mutations prove to be allelic when examined by genetic complementation, then cell division gene *divK* (*divK/pleD*) could play a central role in switching motility on and off at the appropriate times during the cell cycle (see Figure 5).

Mutations in *divL* and *divJ* also suppress the defects in motility and stalk formation conferred by *pleC* mutations, but they restore the mot$^+$ phenotype to only a subset of the *pleC* mutants examined. The *divL* alleles *divL342* and *divL343* displayed a narrow and varied pattern of suppression, particularly when scored for both the mot and div phenotypes at 24° and 37° (Figure 4). This narrow range of suppression is one characteristic of allele specificity that is frequently associated with interaction suppressors. The behavior of the *divJ* alleles is more difficult to interpret because they suppressed the motility defect at 37° of all but two of the *pleC* point mutations examined. At the same time, however, the Cs *div* mutation *divJ332*, which suppressed the motility defect of Tn*5* insertion in *pleC*, was unique in conferring a Ts mot$^-$ phenotype in strains containing the wild-type, *pleC*$^+$ allele (Figure 4).

The ability of *divL* and *divJ* mutations to suppress the motility defects of *pleC301*::Tn*5* (Figure 4) was unexpected, although we have no direct evidence that the Tn*5* insertion is a null mutation. Interaction suppressors are known, however, that suppress null mutations. In *Escherichia coli* suppressors of Tn*10* insertions in *dnaQ* have been mapped to the alpha subunit gene of DNA polymerase III (LANCY *et al.* 1989), and suppressors of deletions in the maltose binding protein gene *malE* have been isolated in the inner membrane protein genes *malF* and *malG* (TREPTOW and SHUMAN 1988). The protein subunits of DNA polymerase III, as well as components of the maltose transport system, are known to physically interact.

Isolation and study of the *divJ*, *divL* and *pleC* gene products will be required to elucidate the mechanism of suppression, but the results obtained here do suggest several different possibilities. Among them are a transient interaction in which the gene products are part of a single transduction pathway that coordinates cell division and polar morphogenesis or, alternatively, a physical interaction between PleC and the cell division gene products. We have proposed previously that targeting of polar structures in Caulobacter depends on organizational centers localized at the cell poles (HUGUENEL and NEWTON 1982), and we speculate that some of the genes identified in this study encode proteins that are laid down at the division site as proposed for proteins in the *E. coli* septalsome (GILL and SALMOND 1987; HOLLAND and JONES 1985). A biochemical analysis will be necessary to explore possible interactions between products of the *pleC*, *divJ* and *divL* genes and their localization within the cell.

A working model for the functional organization of the cell division and developmental genes examined in this study is shown in Figure 5. This scheme, which is independent of the precise mechanism of gene function, proposes that the cell division pathway is interconnected with the *pleC-pleD*-dependent pathway (SOMMER and NEWTON 1989) to control developmental events, including bacteriophage sensitivity, pili formation, cell motility and stalk formation. The suppressor results show that cell division genes *divL* and *divJ* modulate the activity of the *pleC* gene, which is required in turn for formation of φCbK receptors and pili, as well as the activity of *divK/pleD* in regulating motility and stalk formation. *divK/pleD* is placed after *pleC* and shown to be required for regulation of motility because *pleD301* acts as a bypass suppressor of the motility defect, but not the φCbK resistance phenotype conferred by *pleC* mutations.

In summary, we have isolated suppressors in three new cell division genes of *C. crescentus*. Our analysis of these Cs mutations suggests that the cell division and developmental pathways in these bacteria are interconnected and they support a model proposed previously (HUGUENEL and NEWTON 1982) that cell division genes play a direct role in the regulation of polar morphogenesis and differentiation.

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