TRANSFER OF DRUG RESISTANCE FACTORS TO THE DIMORPHIC BACTERIUM *CAULOBACTER CRESCENTUS*

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ABSTRACT

The P-type drug resistance factors RP4, RK2, R702, R68.45, and the N-type drug resistance factor R46 are transferred to *Caulobacter crescentus* at high frequencies. They are stably maintained and their antibiotic resistances are expressed. Experiments with RP4 have shown that intergeneric transfer of RP4 occurs with greater than 90% of the recipient cells in filter matings between *Escherichia coli* donors and *C. crescentus* recipients. Reciprocal matings with *C. crescentus* donors are less efficient, but still occur at a frequency of 10^{-1}. *C. crescentus* strains maintain RP4 as a plasmid, are sensitive to RP4-specific phage, and segregate phage-resistant cells at a frequency of 10^{-4} to 10^{-5}. The RP4 plasmid can be used in several ways: (1) the RP4 plasmid will promote chromosomal exchange between *C. crescentus* strains at frequencies ranging from 10^{-6} to 10^{-8}; (2) RP4 will promote the transfer of nonconjugative colE1 plasmids from *E. coli* to *C. crescentus*; once transferred, the colE1 plasmid is stably maintained under nonselective conditions, can be transferred serially, and segregates independently from RP4; and (3) RP4 can be used to introduce transposons into the *C. crescentus* chromosome, providing the basis for additional genetic techniques.

WE have been involved in a continuing effort to develop genetic techniques suitable for the study of differentiation in the dimorphic bacterium, *Caulobacter crescentus*. Techniques have been developed for the isolation of mutants in the absence of mutagenesis (JOHNSON and ELY 1977) and for generalized transduction (ELY and JOHNSON 1977). In addition, conjugation has been described for *C. crescentus* (NEWTON and ALLEBACH 1975; JOLLICK and SCHERVISH 1972) but this approach is of limited value since few donor strains are available and these strains have been heavily mutagenized. Since antibiotic-resistance factors have been shown to promote chromosomal transfer in a number of organisms (BUKHARI, SHAPIRO and ADHYA 1977) including Caulobacter (ALEXANDER and JOLLICK 1977), we investigated the utility of R-factor-mediated conjugation for genetic analysis of *C. crescentus*. We report here the transfer of a number of drug-resistance factors to *C. crescentus* and present evidence that RP4-mediated conjugation is a versatile system for the genetic analysis of Caulobacter. Furthermore, RP4 can mobilize colE1 plasmids and will be useful for the exchange of genetic material between *C. crescentus* and *E. coli*.

MATERIALS AND METHODS

Bacterial strains: The bacterial strains used in this study are listed in Table 1. NC9303 was isolated by R. C. JOHNSON using a modification of the method of HASHIMOTO and SEKIGUCHI (1976) for hydroxylamine mutagenesis of plasmid DNA. Donor-specific bacteriophages PRD1, PRR1, and ϕGU5 were grown by confluent lysis of J53(RP4) in soft agar overlays on L agar plates. PRD1 and PRR1 were obtained from R. H. OLSEN and ϕGU5 was obtained from J. SCHELL. *C. crescentus* strains were grown as described by JOHNSON and ELY (1977). *E. coli* and *Pseudomonas aeruginosa* strains were grown in L broth (MILLER 1972) or the minimal medium E (VOGEL and BONNER 1956).

TABLE 1

Bacterial strains

Strain	Genotype	Source or derivation
C. crescentus:		
CB15	wild-type	This laboratory
SC120	*his-102*	This laboratory
SC146	*argG105*	This laboratory
SC478	*his-120 flaH135*	This laboratory
SC495	*argG105 ilv-123 rif-103*	This laboratory
SC496	*hisD105 str-103*	This laboratory
SC505	*argG105 asp-108*	This laboratory
SC620	*argG105* (RP4 amp^R kan^R tet^R)	contact of J53(RP4) with SC146
SC621	*argG105* (RP4 *kan*::Tn7)	contact of W3110T$^-$(pRP33) with SC146
SC622	*argG105 zzz*::Tn7(RP4)	contact of J53(RP4) with SC621
SC623	*his-120 flaH135*(RP4)	contact of J53(RP4) with SC478
SC624	*argG105 ilv-123 rif-103*(RP4)	contact of J53(RP4) with SC495
SC625	*hisD105 str-103*(RP4)	contact of J53(RP4) with SC496
SC626	CB15(colE1::Tn5)(RP4)	contact of NC9324 with CB15
E. coli		
C600	*Thr$^-$ leu$^-$ lacY TonA thi$^-$ r$^-$ m$^-$*	L. SHAPIRO
CBK007	*Thy$^-$ ilvA700*::Tn5	C. M. BERG
CU2	*ilvA*	C. M. BERG
DB1287-1	str^R(colE1::Tn5)	D. E. BERG
J53(RP4)	*pro$^-$ met$^-$*(RP4)	H. M. MEADE
J53(R702)	*pro$^-$ met$^-$*(R702 kan^R mer^R str^R sul^R tet^R tmp^R)	P. BARTH
JC411(RK2)	*arg$^-$ his$^-$ leu$^-$ metB lacY* λ^R str^R *sup59*(RK2 amp^R kan^R tet^R)	D. HELINSKI
NC9303	C600(RP4 *Kan$^-$*)	transformation of C600(RP4) treated with hydroxylamine
NC9324	str^R(colE1::Tn5)(RP4 *Kan$^-$*)	contact of NC9303 with DB1287-1
NC9325	*Thy$^-$ ilvA700*::Tn5(RP4 *Kan$^-$*)	contact of NC9303 with CBK007
W3110T$^-$(pRP33)	*Thy$^-$*(RP4 *Kan*::Tn7)	P. BARTH
W3110trpA33	*trpA33*	C. YANOFSKY
χ1781	*his-15 lysA4 arg-32 xyl-2* $T3^R$ $T6^R$ λ^-(R100 cam^R spc^R str^R sul^R tet^R)	R. CURTISS III
S. typhimurium:		
KM302	*hisG46*(R46 amp^R kan^R str^R sul^R tet^R)	B. N. AMES
TR35	*ser$^-$ arg$^-$ his-712*(F'*his*)	P. E. HARTMAN
P. aeruginosa:		
PA025(R68.45)	*leu10 argF10*(R68.45 amp^R kan^R tet^R)	B. W. HOLLOWAY

Bacterial matings: Bacterial matings were performed by one of two methods. When transfer frequencies were high and quantitation was unnecessary, matings were accomplished by streaking overnight cultures of donor and recipient perpendicular to one another across the surface of an agar plate containing medium appropriate for selection of recipient bacteria that had obtained the drug-resistant phenotype. For quantitation and maximum efficiency, 0.5 ml of donor and recipient cultures were mixed, filtered through an 0.45 μm filter 25 mm in diameter, and incubated on nutrient medium for two to seven hr. After incubation, the filters were resuspended in 1 ml of broth and an 0.2 ml aliquot was spread on the appropriate selective medium. When tetracycline was used as a selective agent, concentrations of 0.5 μg per ml and 2.5 μg per ml were used for *C. crescentus* and *E. coli*, respectively. Kanamycin, streptomycin, and trimethoprim were used at concentrations of 25 μg per ml, 10 μg per ml, and 125 μg per ml, respectively. No differences in efficiency were observed when matings were performed with cultures of various cell densities. Since overnight cultures were the most convenient, they were used in subsequent experiments. Transductions were performed using the generalized transducing phage φCr30, as described by ELY and JOHNSON (1977).

Antibiotic resistance: Antibiotic resistance was determined by measurement of zones of inhibition around Difco antibiotic sensitivity discs placed on lawns of the appropriate bacteria.

Observation of pili: Pili were observed in the Siemens Elmiskop IA electron microscope after staining with 2% phosphotungstic acid, as described by JOHNSON, WOOD and ELY (1977).

RESULTS

Transfer of plasmids from E. coli *to* C. crescentus: Since ALEXANDER and JOLLICK (1977) had demonstrated the transfer of the drug resistance factor RP1 to *C. crescentus*, we decided to test a number of additional plasmid-containing *E. coli* strains for the ability to transfer their plasmids to a *C. crescentus* recipient (Table 2). Strains containing the four plasmids from the incompatibility group P or the group N plasmid, R46, transferred their plasmids to *C. crescentus* strain CB15 at frequencies of 10^{-1} to 10^{-2}. These plasmids expressed drug resistance, were stably maintained, and those tested could mobilize the *C. crescentus* chromosome in conjugation experiments (Table 2). In contrast, no His^+ isolates were obtained even when the F'*his*-containing strain TR35 was mated with the *C. crescentus* strain SC121 (*his-102*). Likewise, no Tet^R *C. crescentus* strains were isolated when x1781 containing the F-like factor R100 was mated with CB15. Thus, the F-type plasmids are either not transferred to Caulo-

TABLE 2

Transfer of plasmids from E. coli *to* C. crescentus

Plasmid	Plasmid type	Transfer	Properties of transferred plasmids		
			Kan^R	Tet^R	Chromosome mobilization
RP4	P	+	+	+	+
RK2	P	+	+	+	+
R68.45	P	+	+	+	+
R702	P	+	+	n.d.*	n.d.
R46	N	+	n.a.†	+	+
R100	FII	—	n.a.	n.a.	n.a.
F	FI	—	n.a.	n.a.	n.a.

* n.d. = not determined.
† n.a. = not applicable.

bacter or they are not maintained in Caulobacter. These findings are consistent with the observation that P-type drug-resistance factors tend to have a wide host range, while the F-like plasmids seem to be confined to the enterics (BUKHARI, SHAPIRO and ADHYA 1977) and *Pseudomonas fluorescens* (MERGEAY and GERITS 1978). Since RP4 had the highest frequency of mobilization of the *C. crescentus* chromosome in preliminary experiments, we chose it for further study.

Transfer of RP4 to C. crescentus: RP4 is a P-type plasmid that carries genes coding for resistance to the antibiotics tetracycline, kanamycin, and ampicillin. Qualitative transfer of RP4 from *E. coli* to *C. crescentus* can be accomplished by streaking overnight cultures of the donor and recipient strains perpendicular to one another on restrictive media. In order to determine the frequency of this transfer, donor and recipient cultures were filtered together and incubated prior to dilution and plating, as described in MATERIALS AND METHODS. In a two-hour mating, more than 95% of the *C. crescentus* recipient cells received the RP4 plasmids from the *E. coli* donor strain J53(RP4) (Table 3). Similar frequencies were obtained when J53(RP4) was mated with *E. coli* recipients. In reciprocal experiments using the *C. crescentus* strain SC620 (*argG105*(RP4)) as donor, matings were ten- to 100-fold less efficient, with the frequency of transfer continuing to increase as the time for the mating increased.

Expression of RP4 in C. crescentus: *C. crescentus* clones harboring RP4 became resistant to both tetracycline (Tet^R) and kanamycin (Kan^R), indicating that the genes coding for resistance to these two antibiotics are expressed in the *C. crescentus* cytoplasm (Table 4). Expression of the gene coding for ampicillin resistance (Amp^R) could not be determined since our wild-type strains of *C. crescentus* are resistant to penicillin (JOHNSON and ELY 1977). In addition to the drug resistance, the strains harboring RP4 synthesized sex pili that appeared identical to those synthesized by the *E. coli* strain J53(RP4) when viewed in the electron microscope. These pili were scattered over the surface of all the *C. crescentus* cell types and were larger in diameter than the vegetative pili, which are found only at the flagellar pole of swarmer cells. Thus, RP4 appears to be

TABLE 3

Frequencies of transfer of RP4 in crosses between E. coli *and* C. crescentus

Donor	Recipient	No. recipient cells recovered ($\times 10^7$) Tet^R	Total	Frequency of transfer*
J53(RP4) (*E. coli*)	W3110*trpA33* (*E. coli*)	102	108	95%
J53(RP4) (*E. coli*)	CB15 *C. crescentus*)	38	38	100%
SC620 (*C. crescentus*)	CB15 (*C. crescentus*)	84	657	13%
SC620 (*C. crescentus*)	W3110*trpA33* (*E. coli*)	51	456	11%

* Percentage of recipient cells that are Tet^R after a two-hour mating. Recipient cells were identified by replication into minimal medium for CB15, or minimal medium supplemented with tryptophan for W3110*trpA33*.

TABLE 4

Expression of RP4 genes

Host	Amp^R	Kan^R	Tet^R	ϕ^{S*}	ϕ production†	RP4 pili
E. coli	+	+	+	+	+	+
C. crescentus	n.d.‡	+	+	+	—	+

* Sensitivity to RP4-specific phages PRD1, PRR1, ϕGU5 in complex media.
† Phage production after infection with PRD1, PRR1, ϕGU5 in complex media.
‡ n.d. = not determined, since wild-type *C. crescentus* is Amp^R.

capable of synthesizing its sex pilus in Caulobacter in the same manner as it does in *E. coli* and there is no specificity with regard to location or cell type as is found with the *C. crescentus* vegetative pili.

Additional evidence for normal RP4-specific pilus expression in Caulobacter is the fact that *C. crescentus* strains harboring RP4 become sensitive to the donor-specific phages PRD1, PRR1, and ϕGU5. All three of these phages have been shown to adsorb to RP4-coded pili in *E. coli* (BRADLEY and COHEN 1977; OLSEN and SHIPLEY 1973; ELY and SCHOENLEIN, unpublished) and, consequently, sensitivity to the phage is indicative of the presence of RP4-specific pili. Lysates of each phage formed zones of lysis when spotted onto lawns of RP4-containing *C. crescentus* strains, but had no effect on strains lacking the plasmid. Despite the lysis, the phage did not form plaques and could not be propagated on any Caulobacter strain. Spontaneous segregants having lost the RP4 plasmid were resistant to the phage and could be obtained at a frequency of 10^{-4} to 10^{-5} from within the zones of phage lysis. Alternatively, antibiotic-sensitive isolates could be obtained after serial subculture, since doubling times were 50% longer for strains containing RP4.

Mobilization of colE1 by RP4: In addition to transferring itself, RP4 was shown to be capable of mobilizing the nontransmissable plasmid colE1 and transferring it to a *C. crescentus* recipient. An RP4 *Kan*$^-$ plasmid from NC9303 was introduced into DB1287–1, which harbored a colE1::Tn5 plasmid containing a gene coding for resistance to kanamycin, by selection for tetracycline resistance. The resultant strain NC9324 was crossed with CB15 and plated on minimal medium supplemented with trimethoprim and kanamycin (the trimethoprim selects against the donor strain, since *E. coli* is sensitive to trimethoprim while *C. crescentus* is resistant to the drug). *C. crescentus* clones that were Kan^R were obtained at a frequency of 10^{-6}, indicating that the colE1::Tn5 plasmid had been transferred. One of these clones, SC626 was purified for further analysis. SC626 was found to be Tet^R, indicating the presence of the RP4 *Kan*$^-$ plasmid, and maintained both Tet^R and Kan^R in the absence of selection. Kan^S and Tet^S isolates were obtained by screening after serial subculture. The majority of the Tet^S isolates were still resistant to kanamycin, indicating that the colE1::Tn5 plasmid can be maintained in the absence of RP4. Similarly, most of the Kan^S isolates were Tet^R, demonstrating again that segregation was independent of the presence of RP4.

Serial transfer of the colE1::Tn5 plasmid was shown by crossing SC626 with *E. coli* strains C600 and CU2 on E media supplemented with the required amino acids and kanamycin. Since *C. crescentus* will not grow on E media due to the high salt concentration, the only Kan^R clones should be derived from *E. coli* recipients that had received colE1::Tn5. *E. coli* clones resistant to kanamycin were obtained at frequencies of 10^{-5} to 10^{-6}, while Tet^R clones were obtained at frequencies of 10^{-1}. The difference in the two transfer frequencies provides further evidence that the RP4 *Kan*$^-$ and the colE1::Tn5 plasmids exist separately in the *C. crescentus* host and that mobilization and transfer of colE1 occurs with 10^{-4} to 10^{-5} of the successful RP4 transfers.

Chromosome mobilization by RP4: Auxotrophic strains of *C. crescentus* that harbor RP4 gave rise to prototrophs when mated with recipients containing a second mutational marker (Table 5). The matings were performed on filters, as described in MATERIALS AND METHODS, and gave rise to recombinants at frequencies ranging from 10^{-6} to 10^{-8}, depending on the strains involved. Maximum numbers of recombinants were obtained when filters containing donor and recipient bacteria were incubated for three to seven hrs at 33° before resuspension and plating. The recombinant clones obtained from these crosses were stable and did not give rise to auxotrophic segregants when grown on nonselective media. The RP4 plasmid was present in only 20 to 60% of the recombinants. Since RP4 segregates at a much lower frequency (10^{-4} to 10^{-5}), we conclude that RP4 transfer is not a prerequisite for chromosome mobilization. Plasmid-free isolates could be obtained from recombinant clones after selection with the RP4-specific phage ϕGU5. Crosses where both strains harbored RP4, or where both strains lacked RP4, did not give rise to prototrophic recombinants ($< 10^{-9}$). In crosses with an unselected marker, both parental forms of the unselected marker could be recovered (Table 5). Similarly, if more than one unselected marker was

TABLE 5

Inheritance of unselected markers in crosses mediated by RP4

	Donor	Recipient	Selected marker	Unselected marker	Recombinants*
I.	SC623	SC505	His+, Asp+	Arg	15 Arg+ 84 Arg$^-$
II.	SC625	SC495	Str^R, Rif^R	Arg, Ilv, His	19 Ilv$^-$ Arg$^-$ 354 His$^-$ 2 His$^-$ Arg$^-$
			Arg+, His+	Ilv	14 Ilv$^-$ 0 Ilv+
III.	SC624	SC496	Str^R, Rif^R	Arg, Ilv, His	1 Ilv$^-$ Arg$^-$ 83 His$^-$ 1 Arg$^-$ His$^-$ 1 His+ Ilv+ Arg+
			Arg+, His+	Ilv	1 Ilv$^-$ 14 Ilv+

* Approximately 10^8 cells were plated in cross I; approximately 10^9 cells were plated in crosses II and III.

present in a cross, both parental and nonparental combinations of markers could be recovered. Therefore, RP4-mediated conjugation gives rise to chromosomal recombination and can be used to determine the order of markers using reciprocal pairs of three-point crosses.

Isolation of C. crescentus *strains with chromosomal insertions of transposon Tn7*: *E. coli* strain W3110T$^-$(pRP33) has an RP4 plasmid with transposon Tn7 inserted into the *Kan* gene (BARTH and GRINTER 1977). Since Tn7 codes for streptomycin resistance (Str^R) and trimethoprim resistance (Tmp^R), strains harboring pRP33 are Tet^R, Amp^R, Kan^S, Str^R, and Tmp^R. W3110T$^-$(pRP33) was crossed with the *C. crescentus* strain SC146 (*argG105*), and an exconjugant with the appropriate phenotype (SC621) was obtained after selection on minimal medium supplement with arginine and tetracycline. Since only one RP4 plasmid can be maintained in a strain at any one time (BARTH and GRINTER 1977), the introduction of a wild-type RP4 plasmid into SC621 in the presence of kanamycin would result in the loss of the resident pRP33 plasmid. If streptomycin were present in addition to kanamycin, selection would be made for cells that had lost pRP33, but retained the Tn7 transposon. Consequently, when SC621 was mated with J53(RP4) in the presence of kanamycin, approximately 10% of the recipient cells became resistant to kanamycin and sensitive to streptomycin, presumably by replacement of the resident pRP33 by the wild-type RP4. Of the kanamycin-resistant survivors, approximately 0.5% retained the resistance to streptomycin encoded by Tn7. Twelve of these Kan^R Str^R clones were isolated for further analysis. Eleven of the 12 strains transferred Tet^R and Kan^R, but not Str^R, to an *E. coli* recipient. Thus, we conclude that these strains must contain a wild-type RP4 plasmid and that the Str^R coded by Tn7 is not on that plasmid. The 12th strain was unstable and gave rise to drug-sensitive segregants at a high frequency. Since our wild-type strain of *C. crescentus* contains no plasmids (N. WOOD, personal communication; B. ELY, unpublished), we presume that the Tn7 has a chromosomal location in the 11 strains that failed to transfer Str^R. To confirm this conclusion, we grew the *C. crescentus* generalized transducing phage ϕCr30 on three of these isolates and used the resultant lysates to transduce CB15 to Str^R or Kan^R. Since RP4 is about one-fourth the size of the ϕCr30 chromosome (ELY and JOHNSON 1977), it should be too small to be packaged in a ϕCr30 phage particle and, therefore, it could not be transferred by ϕCr30-mediated transduction. In contrast, Tn7 inserted in the chromosome should be transduced as any normal chromosomal gene. In each case, Str^R was transferred, but Kan^R was not. Thus, we conclude that ϕCr30 will not transfer RP4 and that Tn7 was located in the *C. crescentus* chromosome in the three strains.

Interspecies chromosomal transfer between C. crescentus *and* E. coli: SC622 containing both a chromosomal insertion of Tn7 and RP4 was mated with *E. coli* strain W3110*trpA33* on plates containing tryptophan and either streptomycin or trimethoprim. Antibiotic-resistant colonies were obtained from the cross at a frequency of 10^{-5}. They required tryptophan and were resistant to both antibiotics, indicating that Tn7 had been transferred from *C. crescentus* to *E. coli*.

The majority of the 4000 isolates were Amp^R, Str^R, Tet^R, and Tmp^R. In these cases, Tn7 probably was located on the RP4 plasmid without impairing any known plasmid functions. However, approximately 1% of the isolates were sensitive to tetracycline or kanamycin, and we assume that Tn7 was inserted in Tet^R or Kan^R, respectively. Similar frequencies of insertion of Tn7 into the *Tet* and *Kan* genes was obtained by BARTH and GRINTER (1977) in crosses with *E. coli* strains. In addition to the Tet^- and Kan^- insertions, we found insertions of Tn7 into the *Amp* gene (Tn1) at a frequency of 0.2%. This type of insertion has not been described previously. Finally, five isolates were obtained that were Trp^-, Amp^S, Kan^S, Str^R, Tet^S, and Tmp^R. Thus, these isolates had acquired Tn7 (Str^R and Tmp^R) without acquiring RP4 (Amp^R, Kan^R, and Tet^R). Since RP4 was not present and segregates at low frequency (10^{-4} to 10^{-5}), we conclude that Tn7 was transferred to *E. coli* by mobilization and transfer of the *C. crescentus* chromosome, and that it subsequently was translocated onto the *E. coli* chromosome.

Preliminary results from a similar experiment using the Tn5 (Kan^R)-containing *E. coli* strain NC9325 (*thy*$^-$ *ilvA*::Tn5(RP4 *Kan*$^-$) as a donor and *C. crescentus* strain CB15 as a recipient indicate that three of 500 Kan^R Caulobacter isolates have Tn5 inserted into their chromosomes. In this case, Tn5 was transferred to *C. crescentus* by mobilization and transfer of the *E. coli* chromosome. Thus, chromosomal exchange between *E. coli* and *C. crescentus* can proceed in either direction.

DISCUSSION

In confirmation of the report of ALEXANDER and JOLLICK (1977) that RP1 can be transferred to *C. crescentus*, we have shown that four additional P-type drug-resistance factors can be transferred to Caulobacter. The genes for replication, transfer, and antibiotic resistance are expressed in *C. crescentus* and at least three of these plasmids are capable of mobilizing the Caulobacter chromosome. In contrast, plasmids with a narrow host range, such as F and R100, are not transferred and maintained in Caulobacter.

RP4 has been shown to promote chromosome mobilization in *P. aeruginosa* (STANISICH and HOLLOWAY 1971), Rhizobium spp. (MEADE and SIGNER 1977; BRINGER, HOGGAN and JOHNSTON 1978), and *Acinetobacter calcoaceticus* (TOWNER and VIVIAN 1976, 1977; TOWNER 1978), and has been used to construct a genetic map for the latter two organisms. We have demonstrated chromosomal exchange between strains of *Caulobacter crescentus* and construction of a genetic map is underway.

In addition to chromosome mobilization, we have shown that RP4 will mobilize and promote the transfer of colE1 plasmids between *E. coli* and *C. crescentus*. The colE1 plasmids are stably maintained, segregate independently from RP4, and can be serially transferred to other strains. CLARKE and CARBON (1976) have constructed a set of colE1 plasmids that contain random fragments of the entire *E. coli* genome. Since colE1 can be transferred to Caulobacter, it should be possible to transfer *E. coli* chromosomal genes to Caulobacter along with the

colE1 plasmid. Such transfer would allow *C. crescentus* genes to be matched with *E. coli* genes by complementation of the appropriate *C. crescentus* mutants. Also, a similar set of colE1 plasmids containing fragments of *C. crescentus* DNA has been constructed by WEISBORN and SHAPIRO (personal communication), so that the reciprocal experiments should be possible. In addition, we plan to construct a series of RP4 plasmids containing *C. crescentus* DNA to facilitate complementation and *cis/trans* dominance tests.

The introduction of transposons to Caulobacter makes possible a great number of additional genetic techniques. Transposons can be used for mutant isolations, strain construction, genetic mapping, and a variety of other techniques (for review see KLECKNER, ROTH and BOTSTEIN 1977). Such techniques will prove invaluable for the analysis of differentiation in Caulobacter.

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