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Recombination deficient mutant of *Caulobacter crescentus*

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Summary. A recombination-deficient (Rec^-) strain of *Caulobacter crescentus* has been isolated from a collection of mutants sensitive to ultraviolet irradiation. The Rec^- mutant fails to give recombinants following ∅Cr30-mediated generalized transduction or following RP4-mediated conjugation. The recombination frequency in the Rec^- strain is at least 5000-fold lower than in the wild type strains. The Rec^- mutant is indistinguishable from wild type in terms of morphology, growth rate, viability, and phage sensitivities, differing only in properties known to be associated with *recA*-type mutations in other organisms: recombination frequency, ultraviolet sensitivity, and Weigle reactivation. The map location of the *rec-526* allele has not been identified, but *rec-526* can be cotransferred with the *fla-169* mutation by RP4-mediated conjugation at low frequency. This apparent linkage has been used to move the *rec* mutation to other strains. The Rec^- mutant resembles *recA* strains of other organisms and provides a healthy strain severely deficient in recombination for use in complementation and cloning studies involving *C. crescentus*.

Introduction

The Gram-negative bacterium *Caulobacter crescentus* is regarded as a useful model system for studying cellular differentiation (Poindexter 1964; Bender et al. 1980). The *Caulobacter* cell cycle consists of a program of spatially and temporally regulated events terminating in asymmetric cell division. *Caulobacter* is unusual in that these events are intrinsic to the cell cycle and occur throughout exponential growth. The molecular nature of the events that regulate the developmental program is currently being investigated using genetic analysis (Barrett et al. 1982) as well as molecular cloning (Milhausen et al. 1982; Ohta et al. 1984; Purucker et al. 1982). In these analyses, genetic functions are identified by the ability to complement well-characterized mutations. Both of these approaches have been limited by the lack of a *C. crescentus* strain deficient in homologous recombination, since it is time-consuming to distinguish complementation from recombination in Rec^+ cells.

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Mutations in the *recA* gene of *Escherichia coli* virtually abolish homologous recombination and simultaneously cause sensitivity to ultraviolet (UV) irradiation (Little and Mount 1982). Many different bacterial species have genes analogous to the *recA* gene of *E. coli* (Better and Helinski 1983). We assumed that the analogy might extend to *C. crescentus* as well and that among a collection of UV-sensitive (UV^s) mutants we might find a recombination deficient strain. We report here the isolation and characterization of one such UV^s strain of *C. crescentus* in which homologous recombination is reduced more than 5000-fold.

Isolation and characterization of strain CM5256. The wild-type strain CM5000 was mutagenized by irradiation with UV light (30 J/m^2), resulting in a 10^{-3} survival. The irradiated cells were resuspended in PYE broth (Poindexter 1964), incubated overnight at 30° C, diluted, spread on PYE agar plates, and incubated at 30° C until small colonies appeared. Each plate was replicated to two fresh PYE plates, one of which was irradiated with 10 J/m^2 of UV light from a germicidal lamp. Both replicate plates were then incubated at 30° C and examined periodically for colonies that grew on the unirradiated plate but not on the irradiated one. UV sensitive mutants occurred at a frequency of about 10^{-4}.

Strain CM5256 is one of the UV^s strains detected by this method. Strain CM5256 is considerably more sensitive to UV irradiation than its wild type parent strain (Figure 1). The generation time and the ratio of viable cells to turbidity are indistinguishable between strain CM5256 and CM5000 (data not shown). Strain CM5256 is as sensitive to a variety of *Caulobacter* phages as the wild type strain CM5000, since ∅Cbk, ∅Cb5, ∅Cr40, and the generalized transducing phage ∅Cr30 have the same efficiency of plating on both strains (data not shown). Strain CM5256 remains prototrophic and is morphologically indistinguishable from wild type in either phase contrast or electron microscopy (data not shown).

Strain CM5256 is defective in recombination: ∅Cr30 mediated transduction. The recombination efficiency of strain CM5256 was tested by its ability to be transduced to Kanamycin-resistance (Km^r) by a lysate of ∅Cr30 grown on strain SC1028 (*fla-188*::Tn*5*, *str-152*) see Table 1. The *fla-188*::Tn*5* allele simultaneously renders SC1028 non-motile and Km^r. In addition, this particular *fla*::Tn*5* allele results in noticeable filamentation (unpublished observation).

Table 1. Strain list

Strain	Genotype	Source or comments
C. crescentus		
CM5000	+(=CB15)[a]	B. Ely (Poindexter 1964)
CM5210	*uvr-501*	UV mutagenesis of CM5000
CM5219	*uvr-502*	UV mutagenesis of CM5000
CM5234	*uvr-504*	UV mutagenesis of CM5000
CM5246	*uvr-516*	UV mutagenesis of CM5000
CM5248	*uvr-518*	UV mutagenesis of CM5000
CM5252	*uvr-522*	UV mutagenesis of CM5000
CM5256	*rec-526*	UV mutagenesis of CM5000
CM5281	*rec-526 fla-169*::Tn*5* (pVS1)	SC1759 → CM5256[b]
CM5282	*str-501*	Spont. Sm^R of CM5000
CM5283	*fla-169*::Tn*5* (pVS1)	SC1759 → CM5000
CM5284	*proC104 str-502*	Spont. Sm^R of SC451
CM5285	*rec-526 fla-169*::Tn*5* *proC104 str-502* (pVS1)	CM5281 → CM5284
CM5286	*fla-169*::Tn*5* *proC104 str-502* (pVS1)	CM5281 → CM5284
CM5287	*fla-169*::Tn*5* (pVS1)	SC1759 → CM5256
SC451	*proC104*	(Barrett et al. 1982)
SC1028	*fla-188*::Tn*5* *str-152*	(Ohta et al. 1984)
SC1759	*fla-169*::Tn*5* *proA103 str-140* (pVS1)	SC1045 (Ohta et al. 1984)

[a] CM5000 is strain CB15 carried for several years in other laboratories. Growth media and UV irradiation protocols are described elsewhere (Bender 1984)

[b] A → B: Conjugal transfer from A to B mediated by pVS1

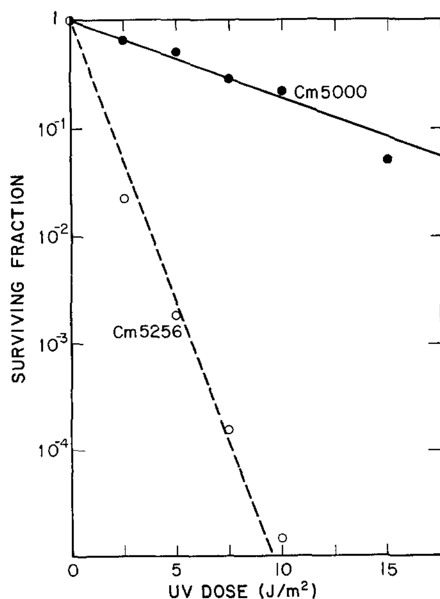

Fig. 1. UV sensitivity of *rec*$^+$ and *rec-526* strains. Cultures of strains CM5000 and CM5256 were irradiated as described (Bender 1984). Samples were withdrawn after the indicated doses and the viable count, determined as colony-forming units on broth plates, compared to the viable count before irradiation (3.5×10^8/ml for strain CM5000 and 7.4×10^7/ml for strain CM5256). •——•, CM5000; o——o, CM5256 (*rec-526*)

Therefore homologous recombinants among the Km^r transductants will be, in addition to Km^r, non-motile and somewhat filamentous, other Km^R transductants are presumed to arise from transposition of the Tn*5* element from the donor transducing fragment onto the recipient chromosome.

When a transducing lysate grown on SC1028 was used to transduce either the wild type strain or any of the UV^s mutant strains listed in Table 1 except CM5256, Km^R transductants arose at equal frequencies (ca. $10^{-7}-10^{-6}$ per cell) and all the Km^R transductants were non-motile indicating homologous recombination at the *fla* locus rather than transposition of Tn*5*. However, no Km^R transductants were observed (<0.1% the wild-type frequency) when the same lysate was used to transduce CM5256. These data suggest that either a) CM5256 is a recombination deficient (Rec$^-$) mutant or b) CM5256 is resistant to the transducing phage ØCr30. Since we had already shown that ØCr30 has an efficiency of plating of 1 on CM5256, we assumed that CM5256 was Rec$^-$ and designated the mutation in this strain *rec-526*.

Strain CM5256 is defective in recombination: RP4 mediated conjugation. It is formally possible that the reduced frequency of transduction observed for CM5256 was the result of some unexpected relationship between the gene mutated and some mechanism necessary for successful transduction. We therefore measured the ability of CM5256 to acquire a *fla*::Tn*5* allele by conjugation.

Strain SC1759 [*fla-169*::Tn*5* *proA103 str104*(pVS1)], like strain SC1028, carries a *fla*::Tn*5* allele (Table 1). This allele allows the distinction between Km^r transconjugants that are homologous recombinants (non-motile) from those that resulted from transposition of the Tn*5* element (motile). Strain SC1759 also carries the *proA103* allele allowing counterselection and the streptomycin-resistance (Sm^r) marker *str-140* which was used here as an unselected donor marker. In addition, strain SC1759 carries the plasmid pVS1 (Barrett et al. 1982), a Km^s derivative of RP4, as the fertility agent promoting RP4-mediated conjugation and chromosome mobilization.

When strain CM5256 was used as recipient in a mating with strain SC1759, Km^r transconjugants were found at a frequency of only 1% of that seen when the wild type strain CM5000 was used as recipient (Table 2). As expected, the vast majority (122 of 129 tested) of the Km^r transconjugants seen when the wild type strain was used as recipient were homologous recombinants (i.e. non-motile). In contrast, a significant portion (28 of 60 tested) of the Km^r transconjugants seen when the mutant strain CM5256 was used as recipient were motile, suggesting transposition of the Tn*5* element rather than homologous recombination at the *fla* locus. Furthermore, all of these motile, Km^r strains (twenty of twenty tested) cotransferred both Km^r and the tetracycline resistance determinant carried on pVS1 at equally high frequency to *E. coli* during a test cross (data not shown). Thus the Tn*5* element responsible for the Km^r phenotype of the motile Km^r transconjugants was presumably located on the pVS1 plasmid.

The remaining 32 Km^r transconjugants from the mating with CM5256 as recipient were non-motile and presumably resulted from homologous recombination. These recombinants were then analyzed for the retention of the *rec-526* allele by testing for the UV^s phenotype associated with *rec-526*. Thirty-one of these thirty-two recombinants were UV^r,

Table 2. Conjugal Transfer of *fla-169*::Tn*5* to CM5256

Recipient	(experiment)	Km^R colonies[a]	Non-motile	UV^R/non-motile	Recombinants[b]	
					Rec^+	Rec^-
CM5000	(1)	2853	114/119	NA[c]	2733	NA
	(2)	2880	8/10	NA	2304	NA
CM5256	(1)	63	10/35	10/10	10	0
	(2)	25	22/25	21/22	21	1

[a] Filter matings (Ely 1979) with 2×10^9 each of donor (SC1759: *fla-169*::Tn*5*, *proA103, str-140*/pVS1) and recipient were plated on minimal medium containing Km and lacking proline. Total Km^R calculated from appropriate dilutions. Several Km^R colonies were purified by single colony isolation, grown in broth, and examined for motility with the phase contrast microscope to distinguish the homologous recombinants (Mot^-) from the Tn*5* mediated transposition events (Mot^+). The homologous recombinants of CM5256 were then tested for UV^s to distinguish the presence of the *rec-526* (UV^s) mutation. The Km^R selection was complicated by the frequent appearance of Km^R variants on minimal medium, even when the antibiotic concentration was raised to 5 mg/ml. This difficulty was overcome by daily monitoring of the selective plates. Colonies resulting from transfer of the Tn*5*-linked Km^R were visible after 3–5 days whereas colonies resulting from mutation to Km^R on this medium were very small until 5–6 days

[b] The recombinants are those Km^R clones arising from homologous recombination (i.e. non-motile) rather than transposition (i.e. motile). The number is calculated from the total number of Km^R exconjugants and the fraction of non-motile clones among the total. Those recombinants which are UV^R are presumed to have arisen in a recombination proficient cytoplasm (Rec^+); those which are UV^s may have originated in a recombination deficient cytoplasm (Rec^-), but see text for caveat

[c] NA, not applicable

suggesting either cotransfer of the rec^+ allele with *fla*::Tn*5* or reversion of *rec-526*. (We have not detected reversion of the UV^s phenotype of *rec-526* strains in over one thousand colonies tested.) Only one recombinant (CM5281) appeared in a UV^s (rec^-) background, in contrast to the more than 5000 recombinants seen in a rec^+ (CM5000) background. Therefore, the recombination frequency seen in strain CM5256 was at most 0.02% of that seen in the wild type background.

The recombination frequency may actually be lower than 0.02% of wild type since the incorporation of *fla*::Tn*5* may have occurred in a cytoplasm that was transiently Rec^+. This possibility arises because the gene defined by *rec-526* may be as much as 10% linked to *fla169*::Tn*5* in pVS1 mediated crosses, as suggested above. If rec^+ were carried into CM5256 along with *fla-169*::Tn*5*, transient expression from the unintegrated fragment might have converted CM5256 into a temporary Rec^+ cell, allowing incorporation of *fla*::Tn*5* with or without the linked rec^+ allele. Alternatively, the unique recombinant seen in the CM5256 background may represent a low-level residual recombination, particularly in light of the observation that the *rec-526* mutation does not totally eliminate Weigle reactivation in *C. crescentus* (Bender 1984). The remaining formal possibility, that the Tn*5* did not recombine but rather transposed into one of the 26 known *fla* loci (Johnson and Ely 1979), is unlikely. Most *fla* mutants are morphologically indistinguishable from wild type. *flaO* mutants tend to filament (O'Neill and Bender, unpublished) and *fla-169* has been mapped to a site in or near *flaO* (B. Ely and A. Newton, personal communication). The recombinant showed a filamentation pattern identical to the *fla-169*::Tn*5* parent and is thus very likely to contain the *fla-169*::Tn*5* mutation of the parental (donor) strain.

Thus two lines of evidence suggest that strain CM5256 is recombination deficient (Rec^-): (1) No recombinants were obtained from ØCr30-mediated generalized transduction even though strain CM5256 is fully sensitive to the transducing phage. (2) Only one recombinant was obtained from conjugal transfer experiments under conditions that gave over 5000 recombinants in a wild type background.

The question of whether *rec-526* identifies a gene analogous to *recA* of *E. coli* remains open. In support of the analogy, *rec-526* causes CM5256 to be very sensitive to UV irradiation. The only recombination gene of *E. coli* whose mutation causes extreme UV sensitivity is *recA* (Clark 1973). Although differences in experimental conditions make comparisons difficult, we found that UV doses resulting in 40% survival of Rec^+ *C. crescentus* resulted in 0.001 to 0.01% survival of strain CM5256. This result might be compared with the original observation of Clark and Margulies (1965) that a UV dose resulting in 40% survival of Rec^+ *E. coli* resulted in 0.003% survival of the Rec^- strains. Furthermore, the only gene of *E. coli* where single mutations result in at least a 10^{-4} reduction in recombination is *recA* (Clark 1973). Finally the *rec-526* mutation reduces the ability of *C. crescentus* to show UV-induced Weigle reactivation of phage ØCbK (Bender 1984); in *E. coli*, the only recombination deficient mutations that eliminate UV induction of W reactivation are *recA* mutations (Witkin 1976). On the other hand, a slight residuum of Weigle reactivation remains in CM5256 and one recombinant was observed at a frequency of 10^{-4} that of wild type. Thus *rec-526* may represent either a *recA* analog with a slight residual activity or an allele of a new *rec* gene different from those seen in *E. coli;* alternatively, recombination and Weigle-reactivation pathways independent of the wild-type function affected by *rec-526* may exist in *C. crescentus*. Isolation of more alleles of this locus and further characterization of *rec-526* will be required to distinguish these possibilities.

Genetic transfer of rec-526. In the mating of SC1759 (*fla-169*::Tn*5*, rec^+) with CM5256 (fla^+, *rec-526*), 31 colonies arose from the apparent cotransfer of rec^+ along with *fla-169*::Tn*5* (Table 2). This suggested the possibility of weak

Table 3. Transfer of *rec-526* from Rec^+ and Rec^- donors to CM5284

Donor (genotype)	Km^R transconjugants/ 2×10^9 donors	Non-motile	UV^s/ non-motile
CM5287 (*fla-169*::Tn*5*)	2425	13/18	NA[a]
CM5281 (*rec-526*, *fla-169*::Tn*5*)	55	42/47	3/42

Filter matings with 2×10^9 each of donor and recipient (CM5284 (*proC104*, *str-502*)) were plated on PYE plates supplemented with both Km and Sm. Total Km^R recombinants were calculated from appropriate dilutions. Several Km^R colonies were tested for motility to distinguish homologous recombinants (Mot^-) from illegitimate recombination by transposon Tn*5* (Mot^+). The Mot^- recombinants were then tested for coinheritance of *rec-526* by scoring UV^s

The use of streptomycin as a counterselection against *C. crescentus* was very effective unless the strain carried a Tn*5* element, in which case Sm^R variants appeared at higher than the frequency expected from spontaneous mutation to Sm^R (ca. 10^{-8} to 10^{-9} in *C. crescentus*). The Tn*5* element encodes resistance to 20 μg/ml of Sm in *C. crescentus* (O'Neill et al. 1984), so high levels of Sm were used (200 μg/ml), the plates were monitored daily, and non-selected markers were used to distinguish Sm^R variants arising after the matings

[a] NA, not applicable

linkage between the *rec* and *fla* genes. To confirm this linkage, strain CM5281 (*fla-169*::Tn*5*, *rec-526*) was mated with strain CM5284 (*proC104*, *str-502*) and the cotransfer of *rec-526* with *fla*::Tn*5*, was tested directly (Table 3). Of 47 Km^r transconjugants tested, 42 were non-motile, indicating that these were recombinant for the *fla* locus. The linkage of *rec* and *fla* was confirmed in that three of these 42 recombinants were UV^s and thus had transferred the *rec-526* allele along with *fla*::Tn*5*. The recombination deficiency of these UV^s recombinants was confirmed by demonstrating failure of ∅Cr30 mediated transduction to generate Pro^+ recombinants in one of these strains (not shown).

The data in Table 3 show also that the Rec^- strain CM5281 as a donor, generated about 40-fold fewer recombinants in RP4-mediated conjugations than did its isogenic Rec^+ sibling. The basis for this difference is unknown. It is tempting to speculate that the *C. crescentus* DNA fragment cloned in the pVS1 plasmid assists in chromosome mobilization by homologous recombination, but Barrett et al. (1982) were unable to detect a difference between RP4-mediated and pVS1-mediated transfer. In any event the data in Table 3 show that homologous recombination is not necessary for chromosome mobilization by RP4-like plasmids, since the Rec^- strain can serve as a donor, or that if recombination is required, the *rec-526* mutation does not entirely eliminate this recombination.

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