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Transposition of Tn7 Occurs at a Single Site on the *Caulobacter crescentus* Chromosome

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Insertion of Tn7 in nine independently derived isolates occurred in a single small region of the *Caulobacter crescentus* chromosome, suggesting that Tn7 insertion occurs at or near a single site. The order of markers in this region of the *Caulobacter* chromosome was shown to be *flaA-argG*-Tn7.

Transposition of Tn7 is extremely site specific in *Escherichia coli*, with all Tn7 transposition events occurring at a single region of the *E. coli* chromosome (2). In contrast, transposition of Tn7 onto the broad host range antibiotic resistance factor RP4 occurs at more than 20 independent sites (3, 4). Similar results were obtained with the Ti plasmid on *Agrobacterium tumefaciens* (9). Recently, Thompson et al. (12) showed that Tn7 inserts into a large number of sites in *Vibrio* sp. strain JT-11 but has a marked preference for a site in the *ilv* gene cluster. Thus, the number of target sites for Tn7 transposition seems to vary tremendously with the target DNA. Preliminary observations of Tn7 transposition in *Caulobacter crescentus* indicated that Tn7 transposed to a limited number of sites on the *C. crescentus* chromosome (6). To determine whether Tn7 transposition occurred at more than one site on the *C. crescentus* chromosome, I analyzed chromosomal insertions of Tn7 by Southern hybridization and by genetic mapping techniques. Results of these experiments showed that the sites of insertion of Tn7 from three isolates were indistinguishable by either technique. Transduction experiments with six additional isolates provided further evidence that there is a single locus for Tn7 insertion.

Nine isolates containing insertions of Tn7 in the *C. crescentus* chromosome were isolated after selection for loss of a Tn7-containing plasmid (6). Each of these plasmids acquired the streptomycin-resistant (Str^r) phenotype encoded by Tn7. Tn7 also encodes trimethoprim resistance, but *C. crescentus* is resistant to trimethoprim (6). The wild-type strain CB15 and three isolates which had been cured of the resident RP4 plasmid were grown to midexponential phase in 10 ml of PYE broth (10), and chromosomal DNA was isolated by the procedure of Wood et al. (13) as modified by J. T. Barrett et al. (1). Approximately 1 μg of each DNA was incubated with 5 U of the appropriate restriction enzyme for 2 h at 37°C and electrophoresed for 2 h at 35 mA in a 1.7% agarose gel in Tris-borate buffer. The gels were blotted to nitrocellulose filter paper (11) and hybridized with nick-translated [^{32}P]thymidine 5′-triphosphate-labeled RP4 *tet*::Tn7 DNA (5). The RP4 *tet*::Tn7 DNA was isolated as described by Barrett et al. (1) from strain NC9321 (*trpA33* [RP4 *tet-106*::Tn7]) (6). Results from these experiments indicated that the wild-type *C. crescentus* did not contain any sequences which hybridized to the probe (Fig. 1, lane D). Three strains, SC644, SC655, and SC656, each containing independent insertions of Tn7 into the *C. crescentus* chromosome, gave rise to an identical banding pattern when RP4 *tet*::Tn7 DNA was hybridized to chromosomal DNA digested with *Bam*HI (New England Biolabs) (Fig. 1, lanes A, B, and C). Similar results were obtained when the chromosome DNA was digested with *Hin*dIII (Bethesda Research Laboratories) or *Bgl*II (Miles) (data not shown). From these results, I concluded that all three Tn7 insertions occurred within the same small region of the *C. crescentus* chromosome.

To verify this conclusion, I attempted to determine the map location of the Tn7 insertions in these strains. For these experiments, conjugational donor strains contained Tn5 insertions in various *che* or *fla* genes scattered around the chromosome (7; Ely et al., unpublished data) in addition to a temperature-sensitive mutation and the kanamycin-sensitive RP4 derivative pVS1 (1). SC644 (*argG105 zzz-103*::Tn7) was used as a recipient. After 4 h incubation on filters (6), crosses were plated at 37°C on PYE plates (10) supplemented with 50 μg of kanamycin per ml to select for transfer of the kanamycin resistance (Kan^r) encoded by Tn5. Kanamycin-resistant temperature-insensitive recombinants were

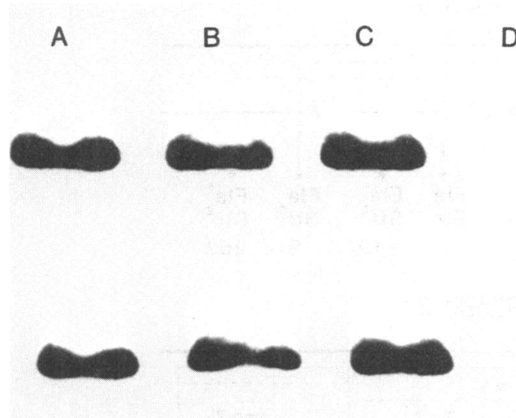

FIG. 1. Hybridization of ^{32}P-labeled RP4 *tet-106*::Tn*7* DNA to nitrocellulose blots of *Bam*HI-digested *C. crescentus* chromosomal DNA. (A) SC656 (*argG105 zzz-102*::Tn*7*) DNA, (B) SC655 (*argG105 zzz-101*::Tn*7*) DNA, (C) SC644 (*argG105 zzz-103*::Tn*7*) DNA, and (D) CB15 DNA. The bands shown in lanes A through C represent fragments, including the Tn*7*-chromosomal junctions, and are approximately 4 and 18 kilobases. The small internal fragment obtained from a *Bam*HI digestion of Tn*7* would not be observed on this gel.

screened for motility (Fla), streptomycin sensitivity (Str^s), and the arginine requirement (Arg). When *fla-185*::Tn*5* was the selected marker, the Str^r associated with Tn*7* was lost 96% of the time. Revertants of the recipient strain to Kan^r were identified by the absence of the associated motility marker and were not included in the analysis. The Str^s colonies in this cross were all Arg$^+$. This result was consistent with our earlier observation that *fla-185* was closely linked to *argG* (R. Croft and B. Ely, unpublished data). However, the fact that we observed 100% cotransfer of Tn*7* and *argG* indicated that these two markers were extremely close to each other.

Since markers with a high conjugational cotransfer frequency are also cotransferred by transduction (1a), φCr30 grown on SC229 (*flaA104*) was used to transduce SC644 to Arg$^+$ on minimal glucose plates (8, 10). Prototrophic recombinants were screened for motility and for the Tn*7*-associated Str^r. The results of these experiments showed that Tn*7* was 55% linked to *argG105* and 3% linked to *flaA104* (Table 1). Analysis of the data as a three-point cross showed that the most likely order was *flaA-argG*-Tn*7* (Fig. 2, order 1). This order requires fewer crossovers and would be expected to result in recombinant phenotypes in the same proportions as those of the experimental results. The alternative order, depicted as order 2 in Fig. 2, is not likely since Str^s Fla$^-$ and Str^r Fla$^+$ recombinants occurred with approximately equal frequencies. If order 2 were correct, Str^r Fla$^-$ recombinants which require four crossover events should have been considerably fewer than Str^s Fla$^-$ recombinants, which would have been formed by two crossovers. Eight additional Tn*7* insertions were mapped in the same manner and gave rise to similar results (Table 1). Thus, I concluded that in each case the insertion of Tn*7* occurred in a region of the chromosome close to the *argG* gene.

In conclusion, I have shown by two independent criteria that Tn*7* insertions occurred in the same small region of the *C. crescentus* chromosome in nine independently derived strains. Since Tn*7* has a high specificity of insertion and has been shown to insert into a single region on the *E. coli* chromosome (2), the simplest explanation of these results is that Tn*7* insertion occurs preferentially at or near a single site which occurs once on the *C. crescentus* chromo-

TABLE 1. Mapping of Tn*7* insertions by φCr30-mediated transduction

Donor strain	Recipient	Selected marker	Recombinant phenotypes[a]				Linkage of Arg and Str (%)	Linkage of Str and Fla (%)
			Fla$^+$ Str^s	Fla$^+$ Str^r	Fla$^-$ Str^s	Fla$^-$ Str^r		
SC229 (*flaA104*)	SC644 (*argG105 zzz-103*::Tn*7*)	Arg$^+$	253	212	9	7	54	3
SC229 (*flaA104*)	SC655 (*argG105 zzz-101*::Tn*7*)	Arg$^+$	76	64	1	1	60	2
SC229 (*flaA104*)	SC656 (*argG105 zzz-104*::Tn*7*)	Arg$^+$	84	56	2	2	54	2
SC229 (*flaA104*)	SC1772 (*argG105 zzz-113*::Tn*7*)	Arg$^+$	50	41	1	1	53	2
SC229 (*flaA104*)	SC1774 (*argG105 zzz-115*::Tn*7*)	Arg$^+$	94	83	4	0	53	2
SC229 (*flaA104*)	SC1774 (*argG105 zzz-116*::Tn*7*)	Arg$^+$	50	41	1	0	54	1
SC229 (*flaA104*)	SC1176 (*argG105 zzz-117*::Tn*7*)	Arg$^+$	52	43	1	0	55	1
SC229 (*flaA104*)	SC1778 (*argG105 zzz-119*::Tn*7*)	Arg$^+$	53	41	1	0	57	1
SC229 (*flaA104*)	SC1779 (*argG105 zzz-120*::Tn*7*)	Arg$^+$	58	38	0	0	60	0

[a] Arg$^+$ revertants which occurred at a frequency of 10^{-3} per recombinant would have the recipient phenotype Fla$^+$ Str^r.

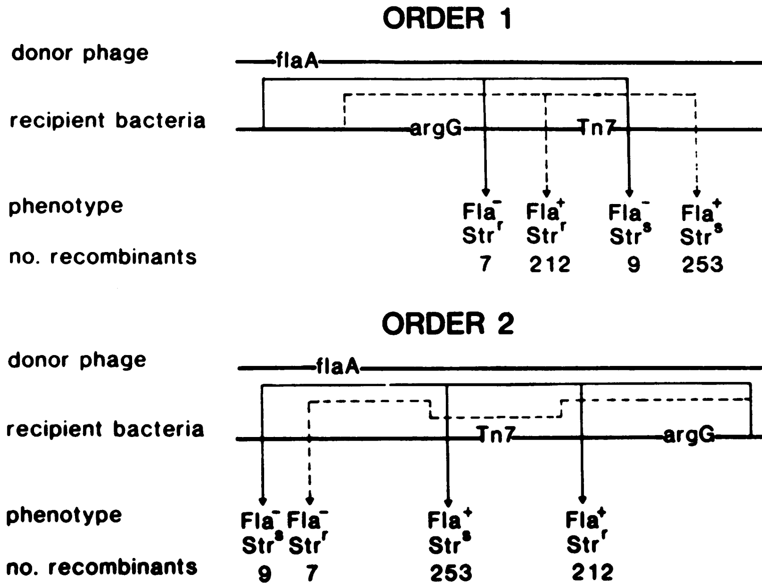

FIG. 2. Mapping of *flaA104*, *argG105*, and *zzz-103*::Tn*7*. Order 1 and order 2 refer to two possible map orders. The third order (not depicted) was ruled out since *flaA* is only 3% linked to each of the other two markers. The phenotype of Tn*7* scored in this cross is streptomycin resistance (Str^r).

some. In contrast, RP4, the Ti plasmid, and *Vibrio* sp. strain JT-11 must contain multiple sites for insertion of Tn*7*.

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