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Chromosomal Rearrangements Induced by Temperate Bacteriophage D108

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As previously shown for mutator phage Mu-1, to which it is closely related, temperate bacteriophage D108 induces chromosomal rearrangements (replicon fusion and transposition of chromosomal segments) in its host genome.

Temperate phage D108 (8) is very closely related to Mu. Physical analysis of the DNA from the two phages shows that although their restriction maps are quite different, homology is such that the DNAs form heteroduplexes on about 97% of their lengths (G. Gill, R. Hull, and R. Curtiss III, Abstr. Cold Spring Annu. Phage Meet. **X**:51, 1977; Toussaint et al., submitted for publication). Like Mu vegetative DNA, D108 vegetative DNA consists of a phage-specific genome linked to random pieces of host DNA on both extremities (Gill et al., Abstr. Cold Spring Harbor Annu. Phage Meet. **X**:51, 1977). D108 also induces mutations by inserting its DNA at random locations in the chromosome of its host (4). It therefore seemed reasonable that, like Mu (10), D108 should induce chromosomal rearrangements in its host's genome.

We tested the ability of D108 to mediate replicon fusion and transposition of chromosomal segments onto a transmissible plasmid upon partial induction of the thermoinducible mutant of D108, D108 *c*ts10 (4). Using the method previously employed with Mu (1), replicon fusion was tested by infecting a partially induced Gal$^-$ *recA* D108 *c*ts10 lysogen grown at 37°C with λ *N r*14 *gal*8, a mutant of the nondefective transducing phage λ *gal*8, which is unable to replicate and to integrate by itself in a *recA* strain (9), and selecting for Gal$^+$ transductants. The D108 prophage was located on an F*lac pro* episome. Three isogenic strains, one nonlysogen, one lysogenic for D108 c^+, and one D108 c^+, D108 *c*ts10 double lysogen, were infected under the same conditions. The results (Table 1) clearly show that D108 *c*ts10 does mediate integration of the λ *gal* at 37°C. The process requires at least one D108 function since it does not occur in those strains that are lysogenic for D108 c^+, which is not induced at high temperature (37°C) and which blocks induction of the D108 *c*ts10 in the double lysogen.

Thirty-two Gal$^+$ transductants were purified and analyzed. We concluded that nine of these carry both D108 *c*ts10 and λ *gal* on the F*lac pro* since, when cured from their episomes, they lost both prophages, and when mated with a Gal$^-$ (D108 c^+) recipient, they transferred simultaneously the episome λ *gal* and D108 *c*ts10. When transferred to a Rec$^+$ recipient, these nine episomes, which were stably Gal$^+$ in the *recA* host, segregated Gal$^-$ derivatives at a frequency of about 1%. The segregants lost all λ *gal* but retained D108 *c*ts10 in their F*lac pro*. This strongly suggests that (as is the case after Mu-mediated integration of λ *gal*, where the λ is surrounded by two Mu genomes in the same orientation) the λ is surrounded by two D108 *c*ts10 genomes similarly oriented, and that these provide homology for excision of the λ *gal* and one D108 *c*ts10 by *rec*-mediated reciprocal recombination. The λ *gal* prophages of the 32 Gal$^+$ transductants were tested for their ability to complement *P*, *R*, *F*, *K*, and *J* mutants of the heteroimmune phage λ i^{434}. Three λ *gal* prophages complemented all of them; 2 complemented all but *J*; 14 complemented *P*, *R*, and *F* but not *K* and *J*; 7 complemented *P* and *R* but not *F*, *K*, and *J*; 3 complemented only *P*; and 1 complemented *R*, *F*, *K*, and *J* but not *P* mutants. Thus, as is the case after Mu-mediated integration of λ *gal*, the λ is integrated in different circular permutations, which leads to inactivation of segments of the λ genome.

The capacity of D108 to mediate transposition of host DNA segments was tested in the following way. A Str^s *recA* (D108 *c*ts10)/F*pro lac* strain was simultaneously induced at 42°C and mated with a Str^r *pro lac thyA recA* (D108 c^+) D108^r recipient for 2 h. Thy$^+$ Str^r sexductants, which should have received an F*lac pro thy* episome, were selected and found at a frequency of $\pm 10^{-4}$ (Table 2). It should be mentioned here that this type of mating between an induced D108 *c*ts10 lysogen and a D108^r (D108 c^+) strain more often fails, except with a few D108^r isolates. Most D108^r strains are resistant on plates but not in broth, as is the case for P1^r strains (Scott, personal communication). Indeed, D108 and P1 host ranges are so far indistinguishable (Kamp,

TABLE 1. *D108-mediated integration of λ N r14 gal8 in recA strains*[a]

Infected host	Frequency of Gal$^+$ transductants
MXR/*F'lac pro*	$<3.3 \times 10^{-8}$
MXR(D108 c^+)/*F'lac pro*	$<7.9 \times 10^{-8}$
MXR(D108 c^+)/*F'lac pro* (D108 *cts*10)	$<10^{-7}$
MXR/*F'lac pro*(D108 *cts*10)	4×10^{-4}

[a] Strains were grown exponentially in L broth + 1% maltose at 30°C up to a concentration of about 2×10^8 bacteria per ml, shifted to 37°C for one doubling, infected with λ *N r14 gal8* at a multiplicity of infection of 10, centrifuged, suspended in the same volume of 10^{-2} M $MgSO_4$, and plated on L agar to measure input viable counts and on 132 minimal medium (3) supplemented with 0.1% Casamino Acids and 0.2% galactose. Plates were incubated for 24 to 48 h at 30°C. MXR (CSH41 [7] made *recA*) is *galE* Δ(*pro-lac*). *F'lac pro* is F'128 (5). D108 c^+ and D108 *cts*10 have been described by Hull et al. (4).

TABLE 2. *D108-mediated transposition of thyA$^+$ onto a F'lac pro episome*[a]

Strain	Frequency of Thy$^+$ Str^r clones
KL132(D108 c^+)D108^r	$<8 \times 10^{-8}$
MXR(D108 *cts*10)/*F'lac pro*	$<5 \times 10^{-8}$
KL132(D108 c^+)D108^r × MXR(D108 *cts*10)/*F'lac pro*	$<1.4 \times 10^{-4}$[b]

[a] Strains to be mated were grown exponentially to a concentration of approximately 3×10^8 bacteria per ml in LB at 30°C and titrated on L agar. A 0.5-ml sample of the donor was mixed with 0.5 ml of the recipient, and as controls 0.5 ml of each strain was mixed with 0.5 ml of LB. The three mixtures were incubated at 42°C for 2 h, centrifuged, suspended in 1 ml of 10^{-2} M $MgSO_4$, plated on 132 medium supplemented with 0.1% Casamino Acids, 20 μg of uracil per ml, 100 μg of streptomycin per ml, and 0.2% glucose, and incubated at 37°C for 36 h.

[b] The frequency of Thy$^+$ Str^r clones in the mating was calculated as the ratio, number of Thy$^+$/input F'. KL132 is *thr leu pro lacY pyrB recA thyA his thi* Str^r (5). MXR, See Table 1.

personal communication; unpublished data). Ten Thy$^+$ Str^r sexductants were purified and further analyzed. All were sensitive to male-specific phage f2, and most of them were Lac$^+$ Pro$^+$. They all contained a D108 c^+ and a D108 *cts*10 prophage since they all produce phages which make clear and turbid plaques at 42°C. The Thy$^+$ marker and the D108 *cts*10 prophage(s) are located on the episome since they were cured and transferred simultaneously with it. When the episome was transferred in a Rec$^+$ recipient, the *thyA*$^+$ marker, which was stable in the *recA* host, segregated at a frequency of about 5%, leaving a D108 *cts*10 on the episome. Thus the *thy*$^+$ marker seems to be surrounded by two D108 *cts*10 genomes in the same orientation, which provides homology for the segregation as has been demonstrated for DNA segments transposed by Mu (2).

In conclusion, for everything that was tested D108 behaved exactly like Mu. It mediated replicon fusion and transposition of host DNA segments, and, like Mu, two copies of the phage genome in the same orientation flank the integrated replicon or the transposed segment. It is likely that D108 *cts*10 also provokes deletions and inversion; to perform all these events it would require a D108 function equivalent to the gene *A* function of Mu. The Mu gene *A* product is a 70K protein (6), the largest protein synthesized by Mu. It is known that D108 synthesizes a protein of the same molecular weight (6). The isolation of large internal deletions in D108 shows that a gene of D108 essential for its transposition maps on the D108 genome at a position equivalent to the Mu gene *A* (Résibois et al., submitted for publication).

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