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Genetics

Acquisition of a determinant for chloramphenicol resistance by coliphage lambda

(recombination/drug resistance determinant/specialized transduction/electron microscopic heteroduplex mapping)

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ABSTRACT **A determinant for chloramphenicol resistance, *cam*, initially detected on a resistance transfer factor (RTF) and since transferred to phage P1, may be acquired from P1 by coliphage λ. λp*cam* are obtained when a λ prophage is induced in bacteria which also harbor P1*cam* prophage. λ*cam* formation is not dependent upon host Rec or λ Red recombination functions.**

Electron microscopic heteroduplex analysis shows that the *cam* locus in two λp*cam*s is a 5% addition of DNA in the *b2* region of λ, not contiguous with *att*. The extent and nucleotide sequence of the DNA insertion in the two independent λp*cam* isolates appear to be the same though they are located at different sites within the *b2* region. We conclude that the determinant for chloramphenicol resistance is contained on a unique piece of DNA which facilitates its insertion into a number of unrelated genomes.

While recombination between homologous sequences of DNA has long been known, recombination between nonhomologous sequences has been appreciated only recently. Phage Mu is a prime example of a DNA sequence that can be inserted into a great number of different sequences (1). The sex factor, F, can have numerous chromosomal locations (2), while phage P2 prefers only a few sites (3). Even phage λ will occupy diverse sites if the strongly preferred site is not present (4). A newer class of genetic elements, called IS (insertion sequence), are able to integrate in many locations (5) and have been found on resistance transfer factors (RTFs) (6), F (7), and the *Escherichia coli* chromosome (8). In many of these cases, the DNA sequence of the inserted element itself is not permuted (5–7, 9, 10). Apparently these recombinations occur between a specific site on the inserted element and a (relatively) nonspecific site on the DNA into which it enters.

This study was undertaken to understand how a drug-resistance determinant, *cam*, is able to recombine with unrelated genomes. *cam* is a gene which codes for chloramphenicol acetyl transferase, an enzyme which inactivates chloramphenicol by acetylation (11). Bacteriophage P1 acquired *cam* from an RTF present in the same cell (12). Subsequently, Scott (13) observed that thermal induction of bacteria lysogenic for both a defective P1 *cam* prophage and a thermoinducible λ prophage yielded plaque-forming λ which carry *cam* (λp*cam*).

We have isolated several independent variants of bacteriophage λ which carry *cam*. Genetic and electron microscopic studies suggest that the *cam* locus is present on a piece of DNA which facilitates its own insertion into unrelated genomes. The acquisition of *cam* is independent of host Rec and λ Red recombination functions.

Abbreviations: RTF, resistance transfer factor; TB, tryptone broth.

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MATERIALS AND METHODS

Phage. λ$^+$ (for a review of λ genetic elements, see ref. 14), λ*c*I857*red*3 and λ*imm*434 were received from M. E. Gottesman; λ*c*I857*bio*69*gam*210*nin*5, from D. Court. The origins of P1kc and P1*cam* were described (15).

Bacteria. The *E. coli* K12 strains used here, their relevant characteristics and references are: N99, nonpermissive (15); N99(λ*c*I857*cam*1); N100, a *recA* derivative of N99 (15); K95, a *nus*1 derivative of N99 (16); HfrH, a streptomycin-sensitive strain; HfrH (*gal-att*λ-*bio*)Δ (4), RW347, a HfrH (*gal-att*λ-*bio*)Δ (λ*c*I857) inserted in *proA* (4); KS10, a HfrH (*gal-att*λ-*bio*)Δ (λ*c*I857) inserted near *cysHPQ* (4), N520, a P1-resistant derivative of AB1157 (15); SA1249, a (*gal-att*λ-*bioA*) deleted strain (provided by S. Adhya); and N2668, a *lig* ts7 strain (17). *Shigella dysenteriae* Sh16 is a λ-resistant, P1-sensitive strain (18).

Strain Construction. λ*c*I857*bio*69*gam*210*nin*5*cam*1 was constructed as follows: λ*c*I857*bio*69*gam*210*nin*5 was crossed with λ*c*I857p*cam*1 and the lysate used to transduce SA1249 to independence of exogenous biotin and chloramphenicol resistance. These transductants were thermally induced and the resulting phage were tested for (*a*) the ability to simulta-

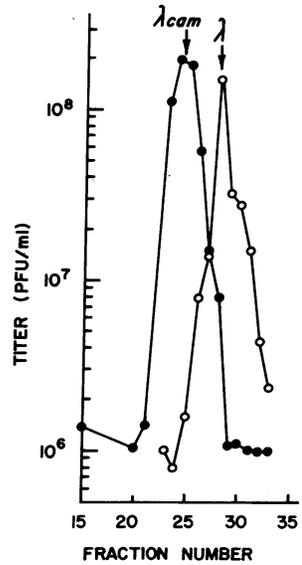

FIG. 1. CsCl density gradient centrifugation of a mixture of λ*c*I857*cam*1 grown by thermal induction of N99 (λ*c*I857*cam*1) and reference phage λ*c*$^+$. The gradient was formed as described in *Materials and Methods* and 75 fractions containing about 0.1 ml each were collected from the bottom of the tube. The fractions were assayed at 40° and clear plaques, due to λ*c*I857 *cam* phage (filled circles), and turbid plaques, due to λ*c*$^+$ (open circles), were counted.

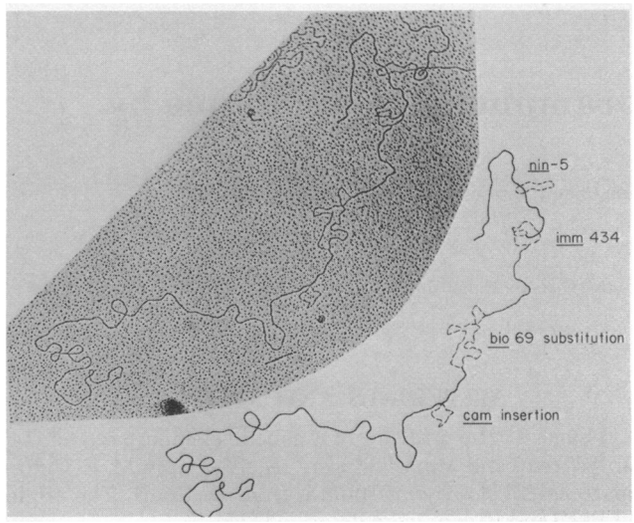

FIG. 2. Heteroduplex formed by DNAs of λcI857*cam*1*bio*-69*gam*210*nin*5 and λ*imm*434. In the interpretive drawing (below), broken lines indicate single-stranded DNA.

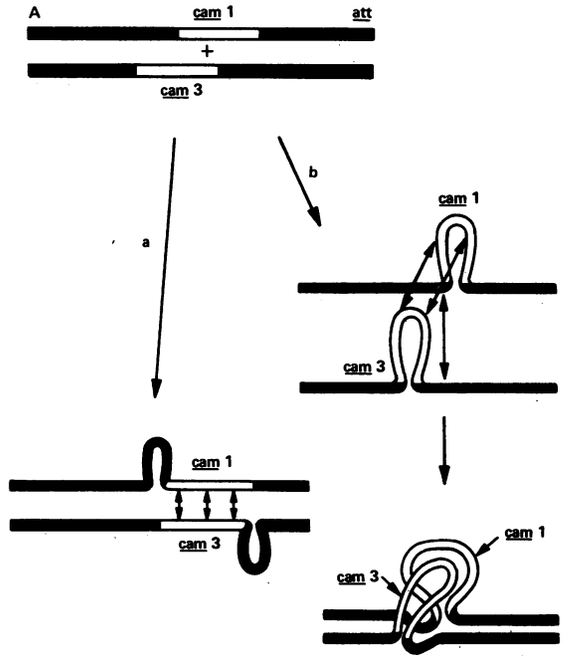

FIG. 4. Models for heteroduplex formation from single strands of λ*cam*1 and λ*cam*3 DNAs. (Only the A to *att* portion of λ is shown for simplicity.) (a) The complementary *cam* DNA sequences pair and the mismatched portions of the λ DNAs loop out. (b) All of the λ DNAs anneal with each other and the *cam* DNAs form loops. Subsequently, the single-stranded *cam* loops interact with each other but form a tangle due to steric hindrance. Double-headed arrows point to complementary sequences.

neously transduce *bio* and *cam*, (*b*) the presence of *nin*5 (ability to form plaques on K95 at 42°), and (*c*) the presence of *gam*210*bio*69 (inability to plate on N100—the Fec phenotype). One such isolate was purified and used here. Bacteria lysogenic for λ*c*I857 derivatives were prepared by isolating thermosensitive bacteria infected with the phage. P1 lysogens were prepared as described (15).

Media. The following media were used: tryptone broth (TB) and TB containing 10 mM $MgSO_4$ and 0.2% maltose (TB–Mg–maltose) (19); LB broth and agar, LB agar containing 12.5 μg/ml of chloramphenicol (LB–chloramphenicol) and LB agar containing 5 mM $CaCl_2$ (LB–Ca) (15). Maltose was from Sigma, chloramphenicol from Calbiochem, polyethylene glycol from Fisher Scientific, and formamide (used undistilled) was from Matheson, Coleman, and Bell.

Preparation, Purification and Assay of Phage Stocks. Bacteriophage λ^+ and λ*imm*434 were prepared on TB plates by the confluent lysis method. Phage stocks used for electron microscopic heteroduplex mapping were purified directly on CsCl gradients. The phage bands were collected from the sides of the tubes to prevent contamination by host DNA. λ*cam* phage were prepared by thermal induction of lysogens, concentrated with polyethylene glycol (19), and banded in CsCl gradients. To prepare the gradients, 4.5 g CsCl were dissolved in 5.8 ml of phage suspension and centrifuged for 16–38 hr at 35,000 rpm in a Beckman Spinco 65 rotor. Phage P1 lysates were prepared by induction or infection as previously described (15). Phage λ was assayed on TB plates with log-phase, TB–Mg–maltose-grown bacteria. P1 was assayed on LB–Ca plates with overnight *Shigella dysenteriae* Sh16 cultures (15).

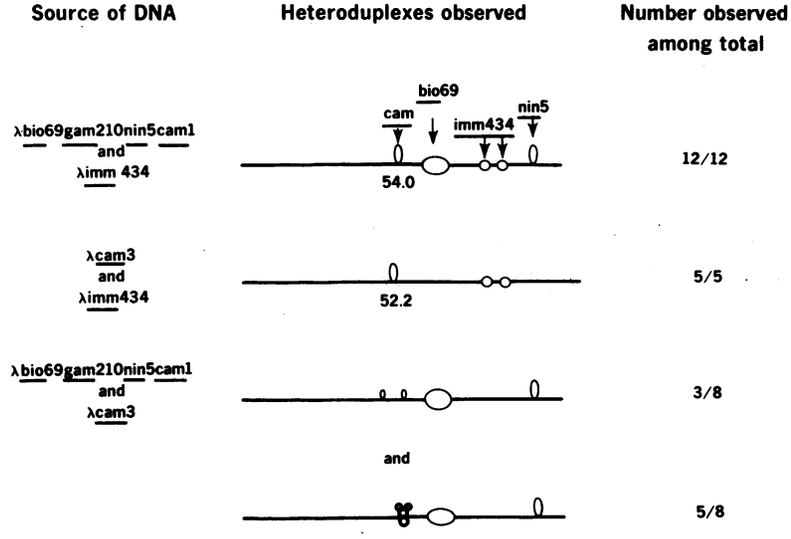

FIG. 3. Schematic depictions of heteroduplex molecules involving DNA from λ*cam*1 and λ*cam*3.

Table 1. Formation of lysates containing λp*cam*

Line	Bacterial component	λc1857		Source of *cam*	Lysate		λp*cam* isolate designation
		Location	Genotype		Produced by	Contains λ*cam**	
1	HfrH	*att*λ	w.t.	P1*cam*	Induction	+	5
2	HfrH(*gal-att*λ-*bio*)Δ	*proA*	w.t.	P1*cam*	Induction	+	3
3	HfrH(*gal-att*λ-*bio*)Δ	*cysHPQ*	w.t.	P1*cam*	Induction	+	4
4	N99	*att*λ	w.t.	P1*cam*	Induction	+	7
5	N99*recA*	*att*λ	w.t.	P1*cam*	Induction	+	8
6	N99	*att*λ	*red*3	P1*cam*	Induction	+	9
7	N99*recA*	*att*λ	*red*3	P1*cam*	Induction	+	10
8	HfrH(*gal-att*λ-*bio*)Δ	—	w.t.	P1*cam*	Infection	—	—
9	HfrH(*gal-att*λ-*bio*)Δ	—	*red*3	P1*cam*	Infection	—	—
10	HfrH	*att*λ	w.t.	R1	Induction	—	—
11	HfrH(P1kc)	*att*λ	w.t.	R1	Induction	—	—
12	N99	*att*λ	*cam*1Δ40	P1*cam*	Induction	+	6
13	HfrH(*gal-att*λ-*bio*)Δ	*proA*	w.t.	P1*cry*	Induction	+†	1

Lysates of λcI857 and derivatives were prepared as follows. For thermal induction, bacteria grown to a concentration of about 2×10^8 cells per ml in TB at 32° were aerated at 40° for 20 min and then aerated at 37° till lysis was complete (usually an additional 30–45 min). To prepare lysates of λcI857 and λcI857*red*3 by infection of HfrH (*gal-att*λ-*bio*)Δ (P1*cam*), it was necessary to cycle the phage two times on the host. First, the λ were plated on the host at 42° and a P1-modified plaque was picked and suspended in Tris–Mg–gelatin (TMG) (15). The second cycle was initiated when the host bacteria, grown at 37° in TB–Mg–maltose, were placed at 40° and infected with the suspended plaque. Lysis was observed after aeration for 3–4 hr at 40°. Lysates were sterilized by treatment with $CHCl_3$ and clarified by centrifugation for 10 min at 10,000 × *g*. Because the λ lysates frequently contained up to 10^4 P1*cam* per ml, the lysates were treated with a one-tenth volume of anti-P1 serum, final titer K = 5, for 30 min at 32°. Equal volumes of lysate and N520 cells suspended in TMG were incubated for 20–30 min at 32° and then plated on LB–chloramphenicol (+ 100 μg/ml of streptomycin sulfate when the lysate was prepared on HfrH derivatives). Phage and bacterial sterility were always tested simultaneously. After 2–5 days at 32°, the plates were scored for chloramphenicol resistance. In each experiment one or two chloramphenicol-resistant bacteria were purified, induced and, in each case, found to produce λp*cam* phage. w.t. = wild type.

* +, Lysate transduces *cam* at a frequency of 1 per 10^6–10^7 PFU. −, Less than 1 *cam* transductant per 10^9–10^{10} PFU.

† From ref. 13.

Determination of the Presence of *cam* on Phage P1 and λ. The presence of *cam* was tested by the method of Kondo and Mitsuhashi (12). Plaques formed on sensitive bacteria at 32° were stabbed with sterile toothpicks and inoculated to LB–chloramphenicol plates at 32° to detect chloramphenicol-resistant lysogens and then to plates containing sensitive bacteria to propagate the phage. It should be noted that, while the plaque derives from a single phage, it is the vegetatively grown progeny that are actually being tested.

Heteroduplex Mapping. Electron microscopic heteroduplex mapping of λp*cam* and derivatives was performed by the method of Davis *et al.* (20) as modified by Gottesman *et al.* (19).

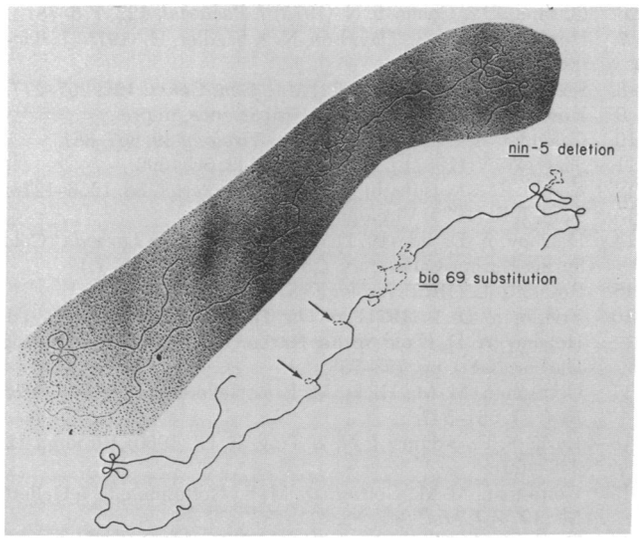

FIG. 5. One type of heteroduplex formed by DNAs of λcI-857*cam*1*bio*69*gam*210*nin*5 and λcI857*cam*3. The arrows point to the single-stranded loops indicated in Fig. 3, line 3.

RESULTS

Structure of two independent λp*cam* genomes

The density of λcI857p*cam*1 (13) was measured by CsCl density gradient centrifugation (Fig. 1). Using the formula of Bellet *et al.* (21), the density of λcI857p*cam*1 is 105% that of λc^+. The size and location of the *cam* DNA present in λcI857p*cam*1 was determined by electron microscopic heteroduplex analysis. When heteroduplexes composed of the DNAs of λcI857p*cam*1 and λ*imm*434 were examined, a single insertion loop containing about 0.05 λ units was seen about 0.20 λ units from the immunity region. For more precise mapping, λcI857*bio*69*gam*210*nin*5p*cam*1 was con-

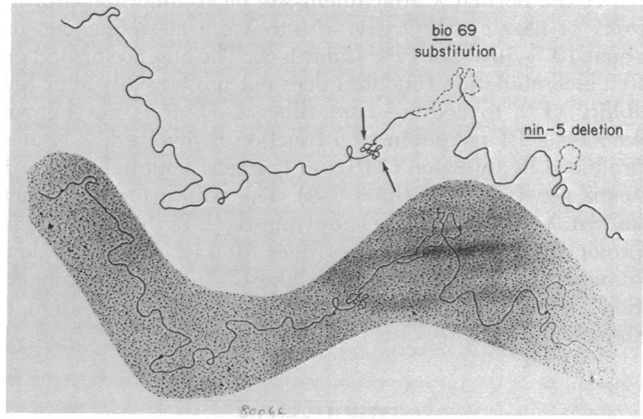

FIG. 6. Another type of heteroduplex formed by DNAs of λcI-857*cam*1*bio*69*gam*210*nin*5 and λcI857*cam*3. Arrows point to tangle of single- and double-stranded DNAs.

structed and heteroduplexes of its DNA with that of λ*imm*434 were examined. As seen in Fig. 2 the *cam*1 insertion is located within the *b*2 region and not at the λ *att* site (defined here by the *bio*69 substitution). Thus, the acquisition of *cam* by λ was not by aberrant excision of a λ located next to *cam*.

The *cam* insertion in the independently isolated λ*c*I857p*cam*3 (see below) appears to be homologous with that of λp*cam*1 but is located at a different site within the λ*b*2 region. Heteroduplexes comprised of the DNAs of λ*c*I857p*cam*3 and λ*imm*434 have one insertion loop of about 0.05 λ units and this loop arises from a point about 0.02 λ units more distant from the immunity region than was found with λp*cam*1. To determine whether the positions and nature of the insertions in λp*cam*1 and λp*cam*3 were similar or different, heteroduplex DNA made from λ*c*I857*bio*69*gam*210*nin*5p*cam*1 and λ*c*I857p*cam*3 was examined. As summarized in Fig. 3, two classes of molecules were seen. One class has a region of complementarity bounded by two small addition loops in the *b*2 region. The region of complementarity between loops is approximately 5% of the length of lambda, agreeing closely with the length of the chloramphenicol addition as estimated from the CsCl density gradient analysis and from the measurements of single-stranded loops. This suggests that the two insertions in λp*cam*1 and λp*cam*3 are very close to the same size and have a base sequence as identical as can be determined by heteroduplex mapping techniques. The single-stranded loops, each approximately 0.015–0.02 λ units in length, are presumed to represent lambda DNA between the sites of the two chloramphenicol insertions (see Fig. 4a). A typical heteroduplex molecule is shown in Fig. 5.

The second class of molecule observed when λp*cam*1*bio*69*gam*210*nin*5 DNA is heteroduplexed with DNA from λp*cam*3 is shown in Fig. 6 and schematically on line 4 of Fig. 3. The tangle of DNA appeared consistently in the same place in the five different DNA heteroduplexes observed. It is presumed that in this structure perfect annealing of all of the complementary λ DNA of the two molecules occurred first; since further pairing of the complementary *cam* sequences requires unwinding the intervening DNA, a tangle of imperfectly paired DNA results (Fig. 4b).

Acquisition of *cam* by λ

The situations in which λ was able to acquire the *cam* locus are summarized in Table 1. Thermal induction of bacteria carrying P1*cam* prophage as plasmid and λ*c*I857 inserted at either the normal λ attachment site or at abnormal sites in *proA* or near *cysHPQ* gave rise to λ*cam* at frequencies of about 10^{-6}–10^{-7} per PFU (lines 1–3). The absence of Red or Rec recombination functions does not substantially alter the ability of λ to acquire *cam* (lines 4–7). It is not known whether a P1 recombination function is involved. λ lysates produced by infection of P1*cam* lysogens contain less than 1 λ*cam* per 10^{10} PFU (lines 8–9). The reason for this is not known. λ did not acquire *cam* from the RTF R1 even in the presence of P1kc prophage (lines 10–11). λ*cam* can lose some of the *cam* DNA (Rosner and Gottesman, in preparation). That this loss does not prevent its reacquisition is shown in line 12.

DISCUSSION

Both the structure of λp*cam* phage and the conditions which lead to their formation suggest that λp*cam* arise by recombination between nonhomologous DNA sequences. In addition, the *cam* determinant contains about 2600 base pairs, which is about five times as much DNA as is required to encode for the 20,000 dalton subunit of chloramphenicol acetyl transferase (22). The fact that the *cam* determinant moves as a unit suggests that it has a special structure. The mobility of the *cam* determinant has also been noted by Iyobe *et al.* (23), who found that *cam* could occupy a number of different chromosomal locations once its parental RTF had been lost. For these reasons we suggest that the *cam* determinant itself codes for a function, or contains a DNA sequence, which facilitates its insertion into unrelated genomes.

Other resistance determinants, *tet* (24), *amp* (25, 26), and *kan* (27), have recently been found to move as a unit from one site on DNA to others. Several of these elements have at one of their ends an inverted repeat of the other end (25–28). In the case of *tet*, the inverted repeated sequences have been identified as IS3 (6), a sequence of about 1400 base pairs (29). A 1400 base pair inverted repeat borders one *kan* insertion of λ (27) and a 130–140 base pair inverted repeat is present on *amp* (25, 26), while no inverted repeat was found for a second *kan* insertion of λ (27). If an inverted repeat is present on the *cam* determinant, our heteroduplex studies indicate that it would have to be shorter than about 100 base pairs. While the significance of inverted repeats is obscure, it is evident that these various determinants possess some mechanism(s) for recombining with diverse DNAs.

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