Transposition of R factor genes to bacteriophage lambda

BERG, Douglas E., et al.

Abstract

Transpositions of segments of R factor (antibiotic resistance plasmids) to bacteriophage lambda have been selected and characterized. Cells of Escherichia coli harboring R factors that determine kanamycin resistance were infected with phage lambda, and lambdakan transducing lines were obtained. Each of the three examined is unusual when compared to lambda transducing phages containing E. coli chromosomal genes: the kan insertions (a) occur at several sites, each well removed from the integration region POP', (b) are not associated with deletion of lambda phage DNA, and (c) are separable from the lambda genome during transduction or during lytic growth. Two insertions from the same R factor contain 1.5 kilobase sequences repeated in inverted order. The properties of the lambdakan phage suggest that R factors contain systems capable of mediating genetic exchange in the absence of extensive DNA homology. It is suggested that such systems of exchange may have played important roles in R factor evolution.

Reference


DOI: 10.1073/pnas.72.9.3628
Transposition of R factor genes to bacteriophage λ

(R factor evolution/inverted repetition/kanamycin resistance/heteroduplex mapping/restriction endonuclease)

DOUGLAS E. BERG, JULIAN DAVIES, BERNARD ALLET, AND JEAN-DAVID ROCHAI

Département de Biologie Moléculaire, Université de Genève, Geneva, Switzerland

Communicated by A. D. Kaiser, June 20, 1975

ABSTRACT Transpositions of segments of R factors (antibiotic resistance plasmids) to bacteriophage λ have been selected and characterized. Cells of Escherichia coli containing R factors that determine kanamycin resistance were infected with phage λ, and λkan transducing lines were obtained. Each of the three examined is unusual when compared to λtransducing phages containing E. coli chromosomal genes: the kan insertions (a) occur at several sites, each well removed from the integration region POP', (b) are not associated with deletion of λ phage DNA, and (c) are separable from the λ genome during transduction or during lytic growth. Two insertions from the same R factor contain 1.5 kilobase sequences repeated in inverted order. The properties of the λkan phages suggest that R factors contain systems capable of mediating genetic exchange in the absence of extensive DNA homology. It is suggested that such systems of exchange may have played important roles in R factor evolution.

The recent widespread use of antibiotics in medicine and agriculture has exerted strong selective pressures on bacterial populations. In the preantibiotic era (more than 2 or 3 decades ago) antibiotic resistance was rare in clinical bacterial isolates; however, today many isolates are resistant to one or more clinically useful antibiotics. It is known that the resistance in the majority of such isolates is due to the presence of resistance plasmids (R factors). Just how R factors have evolved has been a subject of considerable curiosity and speculation. There is an enormous variety of R factors in terms of size (10^7 - 10^9 daltons), and combinations of traits (resistances to a variety of toxic agents, modifications of cell surfaces, capacity for conjugation, etc.). In general, a given R factor shows little or no homology with the chromosome of the bacterium in which it was first detected (9). However, most R factors can exist in a variety of different bacterial species, and it is believed that R factors may have "picked up" their genes from unknown ancestral host species. Since many of the antibiotic-producing species of actinomycetes synthesize antibiotic-modifying enzymes that appear similar to the enzymes responsible for plasmid-borne antibiotic resistance, it has been suggested that actinomycetes may have been one of the ancestral reservoirs from which R factor genes were drawn (3, 4).

As aids to the study of the evolution, and also the function and regulation of expression of the component genes of R factors, we have constructed λ phage derivatives containing small segments of R factor DNA. In starting this work we were encouraged by the knowledge that: (a) phage λ can incorporate into a large number of different DNA sequences, and after induction, give rise to transducing phage lines containing DNA segments that had been adjacent to the site of phage insertion (3); (b) phages (P1 and P22) containing R factor genes have already been obtained (6-8); (c) certain segments of R factors, for example, the ampicillin resistance, ampr, segment of plasmid RP4, can be transposed to other replicons (9). We anticipated that the nature and function of R factor DNA segments cloned in λ phage genomes would be easily studied; many genetic and physical techniques are available for analyzing λ genomes, and the biology of the phage is well known (10).

The three λR hybrid phage lines we describe here carry genes encoding resistance to kanamycin as insertions at sites in the λ genome well removed from the integration region POP'. The behavior and structure of these phages suggest that genetic exchange events, many of which may be similar to the previously noted transposition of ampr, may have played an important role in plasmid evolution by facilitating the "pick-up" of antibiotic resistance and other genes from ancestral hosts. λR phages prepared in vitro may provide valuable tools for understanding these exchange events.

MATERIALS AND METHODS

Phage and Bacterial Strains. Phage λbb is λb515 b519 c1857 Sam7 (from R. Weisberg), contains two small deletions to the left of attλ (Fig. 1), is lysogenic defective in bacterial strains that are not suIII', and is thermostable. λ1857 Sam7 was used as the reference "wild-type" phage for physical measurements. λimm21 phages carrying the amber mutations O8, F3, Q117, A121, G9, and J17 have been described previously (11).

Escherichia coli K12 strains were DB729, su' attλ' BOB' Δ; DB594 attλ BOB' Δ; DB973 suIII'; CB0129, thy' leu' suII'; R factor plasmids JR67 (Ism, SmR, KanR) and JR72 (F', ChR, CmR, HgR, KanR, SmSpR, TeR) from the collection of J. Davies, were introduced into DB729 by conjugation.

Media. Tryptone broth contains 10 g of tryptone and 8 g of sodium chloride per liter; L broth contains 10 g of tryptone, 5 g of yeast extract, 0.5 g of sodium chloride per liter, adjusted to pH 7 with NaOH. Solid media were prepared from the above by the addition of 1% agar. Where needed, glucose (0.2%), maltose (0.2%), and kanamycin (20 μg/ml) were added.

Isolation of λR Hybrid Phage. This procedure is a modification of the procedure of Shimada et al. (5). E. coli strains DB729/JR67 and DB729/JR72 were grown to stationary phase in tryptone broth, infected with λbb at a multiplicity of 10 phage per cell at 30°C, diluted 20-fold into 200 ml of tryptone broth, and grown with aeration at 30°C to stationary phase. The phage lytic cycle was initiated as follows: a 10 ml
Electron Microscope Heteroduplex Mapping. Heteroduplexes were prepared by denaturation and renaturation of purified phage DNA essentially as described by Davis et al. (12) using a DNA solution containing 40 mM Tris, 4 mM EDTA (pH 8.5), 40 μg/ml of cytochrome c in 50% formamide spread on a hypophase containing 1 mM Tris, 0.1 mM EDTA (pH 8.5) in 20% formamide. DNA molecules were examined in a Philips EM300 microscope, and the negatives were traced on a Nikon profile projector and lengths were measured with a Numonics electronic graphic calculator. Five pSC101 DNA molecules in the vicinity of the heteroduplex were measured and the average was used as an internal length standard. The length ratio between pSC101 and λ DNA was found to be 0.190. A λ DNA molecule has 46.5 kb (kilobases) and pSC101 DNA contains 8.84 kb (13, 14).

Restriction Endonuclease Digestion. Specific endonucleases were prepared and used as described previously using 1% agarose gel electrophoresis for analysis (15). The buffer used for electrophoresis was 20 mM Na acetate, 40 mM Tris acetate, pH 8.

RESULTS

Phages capable of transducing kanamycin resistance were obtained from lysates of R-factor-carrying strains infected with λbb as described in Materials and Methods. These phages will be referred to as λbb kan phages. λbb kan-1 and λbb kan-2 were obtained from E. coli DB729/JR67 and λbb kan-3 was obtained from E. coli DB729/JRT2. These transducing phages have been studied and characterized in several ways.

They are not defective; single phages form plaques which at 30° contain lysogenic kan R bacteria in the plaque centers. The lysogens are resistant to neomycin and kanamycin, and cell-free extracts contain the expected neomycin-kanamycin phosphotransferase activities (D. I. Smith and J. Davies, unpublished).

In preparing stocks of λbb kan-3 from single plaques we noticed that a high proportion of the progeny phage were no longer capable of transducing Kan R. These derivatives of λbb kan-3 have lost DNA, probably the kan genes, since they, unlike the parental λbb kan-3, are resistant to treatment with 0.01 M EDTA (pH 8.5) at 37° for 10 min. (Ref. 16 shows how resistance to chelating agents is a measure of the size of A phage genomes.) In contrast, λbb kan-1 and λbb kan-2 are quite stable during lytic growth (Table 1A).

The Kan R transductants resulting from λbb kan-3 infections were all lysogenic (Table 1B).

In the cases of λbb kan-1 and λbb kan-2, Kan R transductants were λ lysogens provided that strain 594 (attλBOB') was used as the recipient. By contrast, however, most Kan R transductants of strain DB729 (attλBOB') were not λ lysogens, by the criteria of lack of immunity or of any of seven λ phage genes (Table 1B). Approximately 1% of the transductants exhibited new growth requirements. Thus, kan-1 and kan-2 are transposable from λ to other replicas such as the E. coli chromosome. Apparently similar phenomena involving transposition of other R factor genes have been studied independently by others (9, 17–19).

The λbb kan DNA's were examined by electron microscopy. Single DNA strands, and also heteroduplexes between λbb kan-1 and λbb kan-2 showed prominent "foldback" (double-stranded stem plus single-stranded terminal loop) structures at positions 70.7 and 76.1, respectively, on the λ physical map (Fig. 1a and b; Table 2). Heteroduplexes of...
FIG. 1.  (a) Single stranded λbb kan-1 DNA molecule displaying snapback region “S” (between the two arrows) and the single-stranded loop “l”. (b) Heteroduplex of λbb kan-2/λ: “S” and “l” denote the same as in (a). The small circles are pSC101 molecules. The single-stranded loops corresponding to the b515 and b519 deletions are indicated. (c) Heteroduplex of λbb kan-3/λ. The single-stranded loop corresponding to the kan-3 segment is indicated. (d) Homoduplex of λbb kan-1/λbb kan-1. Arrow shows insertion point of the two stem regions into λ; Λ indicates the partially annealed loops. (e) Heteroduplex of λbb kan-1/λbb kan-2. Arrows show the two insertion points of the kan-1 and kan-2 fragments; Λ indicates the partially annealed loops.

λbb kan-3/λ show an insertion at 65.0 and no foldback structures were seen in single DNA strands or in heteroduplexes (Fig. 1c).

The heteroduplexes were examined for the presence of single-stranded DNA loops opposite the kan-1, kan-2, and kan-3 insertions; none was found. The absence of such loops
Fig. 2. Endonuclease digests of DNA from phage $\lambda$, $\lambda$bb, $\lambda$bb kan-1, $\lambda$bb kan-2, and $\lambda$bb kan-3. Digestion, separation, and identification of the fragments on agarose gels was carried out as described previously. Reading from the right, samples 1–5 were digested with endonuclease HindIII, and samples 6–10 were digested with endonuclease EcoRI. The samples are: (1) $\lambda$, (2) $\lambda$bb, (3) $\lambda$bb kan-1, (4) $\lambda$bb kan-2, (5) $\lambda$bb kan-3, (6) $\lambda$, (7) $\lambda$bb, (8) $\lambda$bb kan-1, (9) $\lambda$bb kan-2, and (10) $\lambda$bb kan-3. In the HindIII digest of $\lambda$bb (sample 2) (1) the slowest moving fragment includes genes for the late region of the phage, (2) the next fragment (lower molecular weight) extends from a site in the b2 region to a site in the immunity region, (3) the third fragment contains genes to the right of the immunity region, (4) the fourth (faint) fragment contains the right end of linear $\lambda$ DNA and (5) the fifth (fastest moving) fragment contains part of the b2 region, between fragments (1) and (2). In the EcoRI digest of $\lambda$bb (sample 7), (1) the slowest moving band contains the DNA of the late genes, (2) the faster moving band contains the immunity region, (3) the next band includes the nin region, (4) the fastest moving band, forming a doublet with band (3) contains genes to the left of the immunity fragment. A band of molecular weight $2.3 \times 10^6$ is not seen in any of the EcoRI digests of $\lambda$ DNA because it is associated with fragment 1 through "sticky end" homology.

indicates that insertion of the kan DNA segments into the $\lambda$ genome was not accompanied by significant deletion of any portion (i.e., more than 100 base pairs) of the $\lambda$ genome. The $\lambda$kan-1 and $\lambda$kan-2 insertions are probably the same DNA segment of the ancestral plasmid JR67, although inserted into different regions of the $\lambda$ genome. Their lengths are the same (stem = 1.46 kb; loop = 2.32 kb) (Table 2), and a similar structure with the same dimensions in stem and loop regions is found in single DNA strands of the ancestral plasmid JR67 (unpublished). The terminal loops in homoduplexes, and in $\lambda$bb kan-1/kan-2 heteroduplexes are partially annealed with each other (11 of 12, and 17 of 24 molecules scored, respectively) (Fig. 1d and e). Such annealing is not seen with any other single-stranded DNA segments. The double strandedness of the stem region in $\lambda$bb kan-1 and $\lambda$bb kan-2 indicates that the R factor DNA sequences at one junction with $\lambda$ DNA are repeated (in inverted order) at the other junction. We interpret the partially annealed terminal loops as being completely homologous; partial annealing would occur when the intramolecular association of complementary sequences in the stem region precedes the intermolecular reaction between the loops.

Digestion of the transducing phage DNAs with specific endonucleases confirmed and extended the conclusions obtained from electron microscopy. As shown in Fig. 2, the gel electrophoresis patterns of EcoRI fragments of $\lambda$bb kan-1 and $\lambda$bb kan-2 were indistinguishable, with the exception that fragment 2 was replaced by a slower moving (higher molecular weight) fragment. This clearly indicated that $\lambda$bb kan-1 and $\lambda$bb kan-2 contain extra DNA added between the map positions 66.2 and 80.9 (Fig. 3), and that this extra DNA is not cleaved by EcoRI. The size of the fragments inserted into the two phages is identical, which is in agreement with the data obtained by electron microscopy.

From the analysis of EcoRI fragments of $\lambda$bb kan-3 we deduced that this phage contained an insertion in EcoRI fragment 4, between map positions 54.6 and 66.2, and that this insertion, also, is not cleaved by the EcoRI enzyme.

Digestion of the three transducing phages with endonuclease HindIII fails to generate a fragment (designated 2 in Fig. 2) that extends between map positions 56.8 and 77.5. In the case of $\lambda$bb kan-1 and $\lambda$bb kan-2 three new fragments are produced (compared to $\lambda$bb); one of these fragments is common to both phage DNAs. This indicates that the DNA would occur when the intramolecular association of complementary sequences in the stem region precedes the intermolecular reaction between the loops.

Digestion of the transducing phage DNAs with specific endonucleases confirmed and extended the conclusions obtained from electron microscopy. As shown in Fig. 2, the gel electrophoresis patterns of EcoRI fragments of $\lambda$bb kan-1 and $\lambda$bb kan-2 were indistinguishable, with the exception that fragment 2 was replaced by a slower moving (higher molecular weight) fragment. This clearly indicated that $\lambda$bb kan-1 and $\lambda$bb kan-2 contain extra DNA added between the map positions 66.2 and 80.9 (Fig. 3), and that this extra DNA is not cleaved by EcoRI. The size of the fragments inserted into the two phages is identical, which is in agreement with the data obtained by electron microscopy.

From the analysis of EcoRI fragments of $\lambda$bb kan-3 we deduced that this phage contained an insertion in EcoRI fragment 4, between map positions 54.6 and 66.2, and that this insertion, also, is not cleaved by the EcoRI enzyme.

Digestion of the three transducing phages with endonuclease HindIII fails to generate a fragment (designated 2 in Fig. 2) that extends between map positions 56.8 and 77.5. In the case of $\lambda$bb kan-1 and $\lambda$bb kan-2 three new fragments are produced (compared to $\lambda$bb); one of these fragments is common to both phage DNAs. This indicates that the DNA

![Table 2. Electron microscope determination of position and size of kan DNA segments](image)

The numbers in parentheses refer to the number of molecules measured. Estimate 1 is derived by measurements of heteroduplexes (2 x size of stem region S + 1 x size of single-stranded loop I, cf. Fig. 1a and b). The stem and loop regions were 1.45 ± 0.08 kb (16) and 2.32 ± 0.15 kb (8) for $\lambda$bb kan-1, and 1.46 ± 0.08 kb (27) and 2.32 ± 0.14 kb (8) for $\lambda$bb kan-2, respectively. Estimate 2 is derived by subtraction of the size of EcoRI DNA fragment (2) of $\lambda$ (7.0 ± 0.1 kb) from the size of EcoRI DNA fragment (2) of $\lambda$bb kan-1 (12.9 ± 0.4 kb (7)) and of $\lambda$bb kan-2 (13.0 ± 0.9 kb (33)). Only molecules whose size was within two standard deviations from the mean of the size distribution were considered. DNA molecules were spread and examined as described in Materials and Methods. The single-stranded loops in the heteroduplexes corresponding to the deletions b515 and b519 were used as internal length standards for single-stranded DNA.
fragment inserted into $\lambda bb$ kan-1 and $\lambda bb$ kan-2 is cleaved twice by HindIII. The size distribution of the new HindIII fragments produced from $\lambda bb$ kan-1 and $\lambda bb$ kan-2 is compatible with the conclusion that the same piece of DNA (from JR67) was inserted into both transducing phages, at different sites. HindIII digestion of $\lambda bb$ kan-3 produced only two new fragments that form a doublet; this indicates that the DNA inserted into $\lambda bb$ kan-3 (from JR72) is cleaved once and at a site equidistant from the HindIII sites at map positions 56.3 and 76.2.

Similar analyses with other restriction endonucleases, including Hind, HpaII, and HaeIII have allowed us to locate the various sites of insertion with more precision. The fragments coming from within the kan-1 and kan-2 insertions were identical for each enzyme.

**DISCUSSION**

The $\lambda bb$ kan phages are unusual among $\lambda$ transducing phage lines by three criteria: (1) The foreign (R factor) DNA they carry is inserted into regions of the $\lambda$ genome distant from POP$. (2) The kan segments are not substitutions which replace $\lambda$ DNA. (3) The kan-$\lambda$ association is unstable during lytic growth (kan-3) or during insertion into chromosomes which lack attBOB$'$ (kan-1 and 2). These characteristics make it seem unlikely that the $\lambda bb$ kan phages arose by the usual mode of $\lambda$ transducing phage formation; namely the insertion of $\lambda$ into another DNA molecule by cleavage of the $\lambda$ genome at att$\lambda$, followed by aberrant excision of $\lambda$ from the recipient DNA molecule. The $\lambda$ kan DNA segments may have been transposed to phage $\lambda$ by the insertion of the R factors into $\lambda$, followed by the excision of all R sequences except those of the kan segments. Alternatively, the kan segments may have been transposed directly to $\lambda$ without any R factor-$\lambda$ fusion intermediate.

Transposability may be a general characteristic of genes found on R factors. The apparent identity of kan-1 and kan-2, based on criteria of behavior in transduction, the inverted repeat stem and homologous loop structures, the distribution of restriction enzyme cleavage sites, their similarity to a portion of the ancestral JR67 plasmid, and their sites of insertion distant from att$\lambda$, suggests that kan-1(2) may comprise an intact unit of transposition.

Recent work has uncovered other R-factor-derived antibiotic resistance genes which behave either like kan-1(2) or kan-3. Tc$^\beta$ and Amp$^\beta$ can be transposed to any of a large number of sites on other replicons (9, 17–19). Lambda, like $\lambda$ kan-3, is unstable during lytic growth (L. Rosner, personal communication).

What processes might be involved in transposition? Models may be drawn from our understanding of the insertion–excision systems used by temperate phages such as mu-1, P2, and $\lambda$. Phage DNA is inserted into, and excised from, bacterial DNA by cleavages at special sites on the phage genome. The specificity of this cleavage is governed by the products of genes found very close to the special sites on the bacteriophage chromosome (20). Recently, small DNA elements resembling phage integration gene complexes have been identified in bacterial plasmids and chromosomes. These elements [designated IS (insertion sequence)] insert into genes, and thus cause mutation; several distinct species are known to exist. IS elements are present in several copies in the bacterial chromosome, in R factors, and in related plasmids. They appear to be important in integration of plasmids into the chromosome, pick-up of chromosomal genes by plasmids, and recombination within and among plasmid DNA molecules. IS2 and IS3 are similar in size to the 1.5 kb stem region of kan-1(2) (21, 32).

Regarding the mechanisms used, it seems that plasmid-encoded systems which facilitate transposition and other types of genetic exchange in the absence of extensive homology must play important roles in plasmid evolution; one role would be in the "pick-up" of genes from ancestral hosts. The low frequency of transposition that we have observed may indicate that such exchange events are rare in nature. This might be expected, since the stability of an R factor, and therefore the activity of its exchange systems is subject to natural selection.

We thank Lucien Caro and Pierre-François Spahr for their support and encouragement. This work was supported by grants from the Fonds National Suisse de la Recherche Scientifique 3.810.72, 3.331.74, 3.287.74, and 3.285.74, the Ligue Suisse Contre le Cancer, the Swiss-American Foundation for Scientific Exchange, and an European Molecular Biology Organization Senior Fellowship.