JOURNAL OF BACTERIOLOGY, Mar. 1977, p. 1632-1635
Vol. 129, No. 3

Printed in U.S.A.

Tn402: a New Transposable Element Determining Trimethoprim Resistance That Inserts in Bacteriophage Lambda

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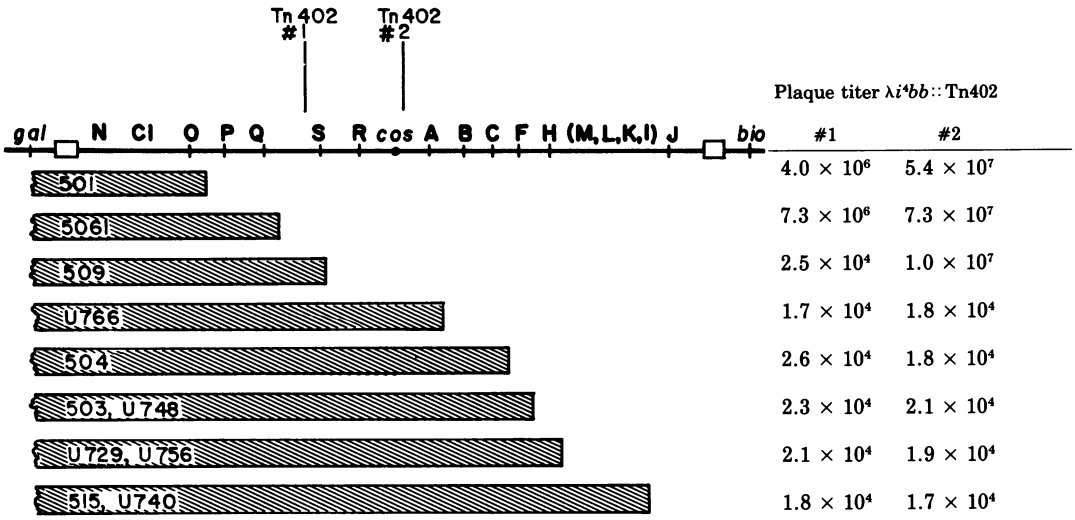
Received for publication 19 October 1976

We have found that the trimethoprim resistance determinant of the IncP plasmid R751 (Jacob et al., 1977; Jobanputra and Datta, 1974) transposes to bacteriophage λ. We call this transposable element Tn402.

A culture of strain JSR26, *pro met*(R751), was lysogenized with λ*imm*434*cts*1*b*515*b*519S7. This phage (hereafter called λi^4bb) has approximately 12% less deoxyribonucleic acid (DNA) than wild-type λ due to the immunity 434 substitution and two deletions in nonessential regions (5, 12). Thus, it can support the insertion of about 5×10^6 to 6×10^6 daltons of DNA without losing the capacity for encapsidation (3). In addition, the phage is thermo-inducible and lysis-defective (1) to facilitate the production of high-titer phage stocks. Two clones of this (R751) (λi^4bb) lysogen were thermally induced, and the resulting lysates were used to infect strain JSR0, *pro met*, at 32°C. The infected cultures were plated on supplemented minimal glucose agar containing 200 μg of trimethoprim per ml (added as a methanol slurry immediately before pouring the plates). Colonies appeared in 48 h at 32°C at a frequency of about 1 per 10^9 infecting phage particles. One trimethoprim-resistant (Tp^r) clone from each infection was grown up and induced. The resulting high-frequency transducing lysates each contained greater than 10^8 Tp^r transducing phage per ml. Thus, we concluded that we had isolated the products of antibiotic resistance determinant transposition into the λ genome, as previously described by Berg et al. (4). The Tp^r phages of independent origin are named λi^4bb::Tn402#1 and λi^4bb::Tn402#2.

Low-multiplicity transductions with both phages yielded some thermosensitive Tp^r transductants that did not produce phage particles. This indicated that both transposition events had inactivated some λ gene(s) essential for lytic growth. CsCl equilibrium centrifugation of the original high frequency transducing lysates confirmed this conclusion. Analysis of the gradients showed that each lysate contained two distinct species: lighter plaque-forming phages (λPFU) without transducing activity and denser Tp^r transducing phages without plaque-forming ability. (The λTp/λPFU ratios in the light and dense peaks were about 10^{-5} and 10^3, respectively.) To determine if the insertion defects affected gene products required for lysogenization, we used CsCl-enriched λi^4bb::Tn402 phage to transduce JSR0 at low multiplicities (down to 10^{-6}) with and without λi^4bb helper phage added at a multiplicity of 3. The level of transduction was always slightly higher without helper. Thus, neither λi^4bb::Tn402 has defective lysogenization functions. This result indicated that the insertion site of Tn402 in each phage lay somewhere to the right of the immunity region (*c1*) on the λ prophage map (Fig. 1). To locate these sites, we infected different prophage deletion strains with CsCl fractions enriched for λi^4bb::Tn402 and looked for rescue of the insertion defect (i.e., an increase in the titer of plaque-forming particles). Figure 1 summarizes the results. The Tn402#1 site is between the end points of the 5061 and 509 deletions, and the Tn402#2 site is to the right, between the end points of the 509 and U766 deletions near the cohesive end (*cos*) site.

We have not so far observed defective λi^4bb::Tn402 prophages to yield full plaque-forming revertants. Thus, it was necessary to confirm the transposon hypothesis (4,6,9) for the origin of trimethoprim-transducing phages and eliminate more complex explanations. We did this by demonstrating that both λTp^r phages contain DNA inserted into a specific site by electron microscope examination of λi^4bb::Tn402/λ*plac*5 heteroduplexes. This method also enabled us to determine an approximate size for Tn402 and detect any special structural features, such as inverted terminal repeats (4,6,9,10). The micrographs in Fig. 2



Plaque titer λi⁴bb::Tn402	
#1	#2
4.0 × 10⁶	5.4 × 10⁷
7.3 × 10⁶	7.3 × 10⁷
2.5 × 10⁴	1.0 × 10⁷
1.7 × 10⁴	1.8 × 10⁴
2.6 × 10⁴	1.8 × 10⁴
2.3 × 10⁴	2.1 × 10⁴
2.1 × 10⁴	1.9 × 10⁴
1.8 × 10⁴	1.7 × 10⁴

FIG. 1. *Deletion map of λ prophage adapted from Shapiro and Adhya (11). The columns at the right give the average plaque titers of peak CsCl-enriched fractions of λi⁴bb::Tn402 #1 and λi⁴bb::Tn402 #2 when plated on different deletion indicator strains. An increase over the background contamination with λi⁴bb PFU (about 2 × 10⁴/ml for both phages) indicates marker rescue. The vertical bars indicate the approximate locations of the Tn402 insertion sites. The map is not drawn to scale.*

are typical of 13 molecules that were analyzed (and others that were not photographed). They clearly show Tn402 inserted into two sites on the vegetative λ DNA molecule: no. 1 at 0.91 fractional distance near the right end of the molecule and no. 2 at 0.02 fractional distance close to the left end of the molecule. These results agree with the prophage deletion map in Fig. 1. There is no evidence for special secondary structure of Tn402 in these grids. An occasional short stalk appears coming out of the duplex at the insertion site, but this is an artifact because we can also see similar structures in some *b*515 deletion loops. Comparison of the single-stranded lengths of Tn402 loops and φX standards (1.9 μm) gives the following sizes for the two independent transposition events: no. 1 = 1.37 φX equivalents and no. 2 = 1.40 φX equivalents.

From these results, we conclude that the R751 plasmid and our transducing phages contain a transposable element of approximately 5 × 10⁶ daltons encoding trimethoprim resistance: Tn402. None of these genetic elements confers streptomycin resistance. Hence, Tn402 is different from Tn7, which determines both streptomycin and trimethoprim resistances and has a molecular weight of 9 × 10⁶ (2). It is possible that one of the transposons (Tn7 or Tn402) is the ancestor of the other. The relationship between these two transposons, details of Tn402 structure, and elucidation of its insertion specificity require further investigation.

We wish to thank Kevin McEntee and George Jacoby for phage and bacterial strains and Louise Chow for the preparation of grids of heteroduplex molecules.

This research was supported in part by a grant from the Louis Block Fund of the University of Chicago and by the National Science Foundation (GB43352 and BMS75-08951).

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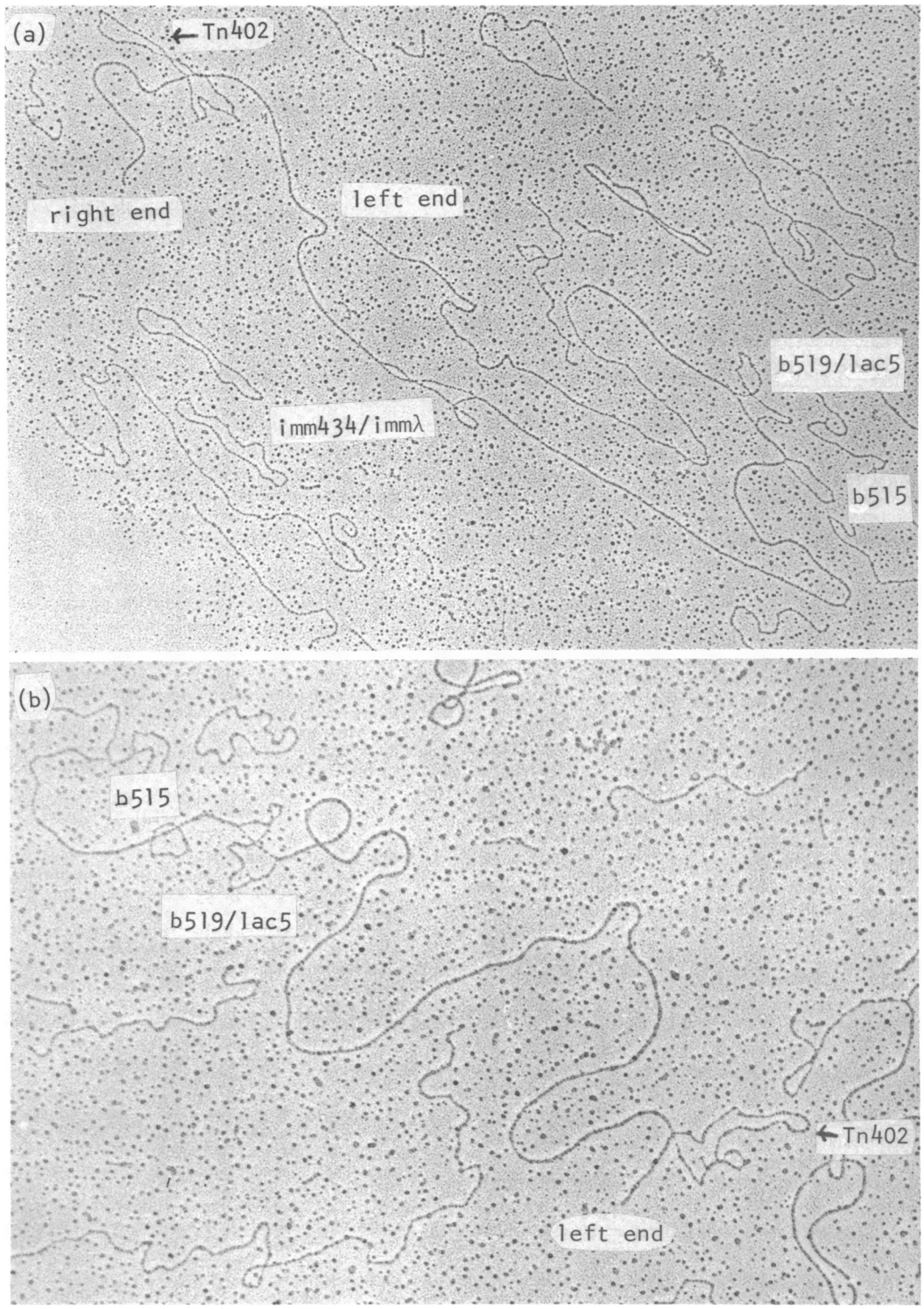
(a)
←Tn402
right end
left end
b519/lac5
imm434/immλ
b515
(b)
b515
b519/lac5
←Tn402
left end

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FIG. 2. *Electron micrographs of λi⁴bb::Tn402#1/λplac5 (a) and λi⁴bb::Tn402#2/λplac5 (b) heteroduplex molecules. High-titer stocks of λi⁴bb::Tn402 were separated from λi⁴bb as a visible lower band in CsCl gradients (25,000 rpm for 36 h at 15°C in the SW50.1 rotor). Phages from these bands were mixed with an equivalent amount of λplac5 DNA (about 5 μg of total DNA), the particles were disrupted and DNA was denatured by alkali, and the mixture was neutralized and allowed to reanneal for 60 min at room temperature in 0.1 M tris(hydroxymethyl)aminomethane ethylenediaminetetraacetic acid (EDTA), and 50% formamide (pH 8.5). The heteroduplex molecules were spread on parlodion grids (the hyperphase contained 0.1 M Tris, 0.01 M EDTA, 45% formamide [pH 8.5], and the hypophase contained 0.01 M Tris, 0.001 M EDTA, 15% formamide [pH 8.5]) and photographed. Internal λ markers (the imm434/immλ and lac5/b519 substitution bubbles and the b515 deletion loop) were used to measure double-stranded distances, and φX circles were used as standards for measuring the single-stranded lengths of Tn402 insertion loops. The length standards used are (i) left cohesive end-plac5 substitution as 0.395 λ equivalents (ii) right cohesive end-imm434 substitution as 0.209 λ equivalents, (iii) imm434 substitution-b515 deletion loop as 0.195 λ equivalents, and (iv) φX double-stranded length of 0.112 λ equivalents (5, 12). The molecule in (b) lacks the right end of λ.*