Bacterial Transposons

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A. Bacterial Plasmids

Bacterial plasmids have been known for more than twenty years and, during this period, one witnessed an extraordinary spread of those extra-chromosomal DNA elements.

Resistance plasmids were first identified in *Enterobacteriaceae* but, later on, have been found in almost every pathogenic bacterial species, including more recently *Hemophilus influenzae* and *Neisseria gonorrheae.*

Plasmids, however, do not only contribute to antibiotic resistance. They can code for many other properties, including heavy metal resistance, production of a cholera-like enterotoxin, tumorigenicity in plants and the capacity to catabolize various substrates. Some plasmids have been found to code for lactose fermentation and this type of plasmid also happens to be of medical concern. Their presence can indeed obscure the detection of pathogens such as *Salmonella* and *Yersinia* since the capacity to ferment lactose is the major key in the sorting of the *Enterobacteriaceae.*

Plasmids consist of covalently closed circular DNA and their essential feature is a replication region that ensures the propagation of the entire structure.

Some plasmids also contain an operon promoting the transfer of the plasmid itself from one bacteria to another one.

B. Translocation of Plasmid Genes

In 1974, Hedges and Jacob observed that the ampicillin resistance determinant of a plasmid called RP4 could be translocated to the bacterial chromosome and various other plasmids genetically unrelated. From one of these derivative plasmids, they could subsequently translocate the β-lactamase gene to a third plasmid. After transposition, the increase in molecular weight was similar in every instance. These authors concluded that a DNA sequence, including the β-lactamase gene, had a specific transposition mechanism and they called it a transposon (now Tn1).

Later on, Berg et al. (1975) observed the transposition of the kanamycin resistance genes from two different resistance plasmids to bacteriophage λ (Tn5 and Tn6).

Gottesman and Rosner (1975) made similar observations with a determinant for chloramphenicol resistance: initially detected on a resistance plasmid

and, later, transferred to phage P1, it could subsequently be translocated from P1 to bacteriophage λ (Tn9).

Still in the same year, Kleckner et al. (1975) reported that a genetic element. 8.3 Kb long, carrying the tetracycline resistance gene was also capable of translocation (Tn10).

Recently, we showed that genes coding for lactose fermentation can jump from pGC1, a plasmid that originated in *Yersinia enterocolitica*, to RPI, a resistance plasmid that originated in *Pseudomonas aeruginosa* (Cornelis et al., 1978) (Fig. 1). This lactose transposon that alters the biochemical phenotype of its host is 16,6 Kb long and has been given the number 951.

Some transposons are listed in Table 1.

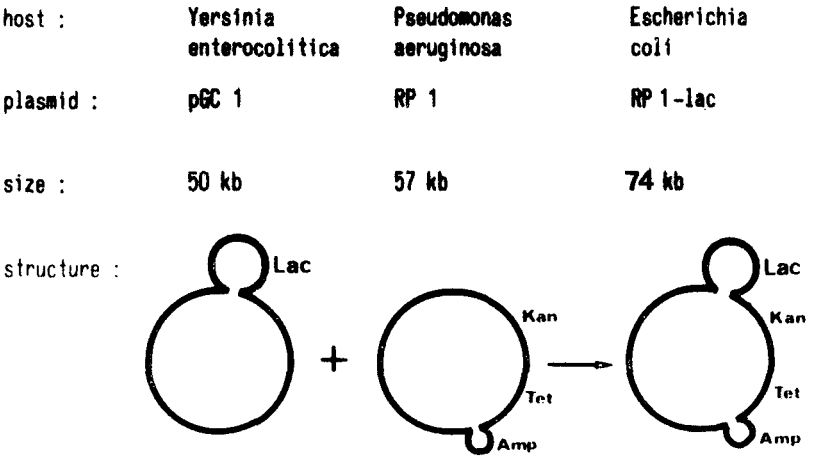

Fig. 1. Schematic representation of the transposition of Tn951. carrying a lactose operon. from pGC1 to RP1. RP1 confers resistance to kanamycine (Km). tetracycline (Tc) and ampicillin (Amp). The latter gene is part of another transposon (Tn1)

Table 1. Some transposable elements

Transposon	Genetical information	Size	References
Tn1, Tn2	β-lactam antibiotics resistance	4,5 Kb	Hedges et al., M.G.G. **132,** 31 (1974) Heffron et al., J. Bact. **122,** 250 (1975)
Tn5, Tn6	Kanamycin resistance	3,8 Kb	Berg et al., P.N.A.S. **72,** 3628 (1975)
Tn7	Trimethoprim and Streptomycin resistance	13,5 Kb	Barth et al., J. Bact. **125,** 800 (1976)
Tn9	Chloramphenicol resistance	2,6 Kb	Gottesman et al., P.N.A.S. **72,** 504 (1975)
Tn10	Tetracycline resistance	8,3 Kb	Kleckner et al., J.M.B. **97,** 561 (1975) Foster et al., J. Bact. **124,** 1153 (1975)
Tn402	Trimethoprim resistance	7,5 Kb	Shapiro et al., J. Bact. **129,** 1632 (1977)
Tn501	Mercuric ions resistance	7,8 Kb	Bennett et al., M.G.G. **159,** 101 (1978)
Tn951	Lactose fermentation	16,6 Kb	Cornelis et al., M.G.G. **160,** 215 (1978)

C. General Features of Transposition

As suggested by Hedges and Jacob (1974), transposons are specific DNA sequences of a precise length.

The size of any replicon suffering transposition does increase and the increment is constant for a given transposon.

Transposition does not require extended homology between the two replicons exchanging the transposon. This is shown in Table 2 for pGCI (*lac*$^+$, *tra* ts, *inc* unknown) and RPI (Amp, kan, tet, *tra*, *inc* P), the two plasmids exchanging Tn951. Thus, transposition cannot involve ordinary recombination occuring after physical breaking and reciprocal exchange of DNA. Accordingly, in some instances at least, transposition turns out to be independant of the *rec* A gene product, the bacterial function involved in such recombination process (Rubens et al., 1976).

Table 2. DNA-DNA hybridization between pGCI and RPI

DNA bound to filter	^{3}H-pGC1 DNA hybridized	
	Exp I	Exp II
pDG1	64	101
pGC1	2300	2303
RP1	74	112
RP1::Tn951	1490	—

Filters were loaded with 2 mcg of DNA. The values given are normalized to 10000 cpm input. pDGI is an unrelated plasmid taken as a negative control. For details, see Cornelis et al., 1978

Transposition is therefore considered as some kind of "illegitimate" recombination involving the termini.

The known transposons can jump on a great number of targets such as different plasmids, bacteriophages and bacterial chromosomes. There seems however to be some kind of specificity: Tn1 for instance was found by Hedges and Jacob (1974) to transpose from RP4 to three different plasmids but not on a fourth one. Bennett and Richmond (1976) also observed that the transposition frequencies of Tn1 could vary 10000 times according to the chosen target. Kretschmer et al. (1977) observed the same phenomenon with Tn3.

Inserted transposons can readily be detected on small replicons such as plasmids and bacteriophages by electron microscopy. When a mixture of two different DNAs sharing homologous regions is denatured and allowed to reanneal slowly, a number of hybrid molecules are generated. If such "heteroduplex" molecules are constructed between a given plasmid and its own derivative carrying a transposon, the structure observed in electron microscopy will be a circular double stranded plasmid carrying a single stranded loop corresponding to the transposon. This kind of analysis clearly demonstrates that transposons are inserted without any loss of DNA from the recipient plasmid.

Moreover, heteroduplex analysis will indicate whether the insertions on two independantly isolated molecules occur at the same site or at two different sites. If the transposon maps at two different loci on the target plasmid,

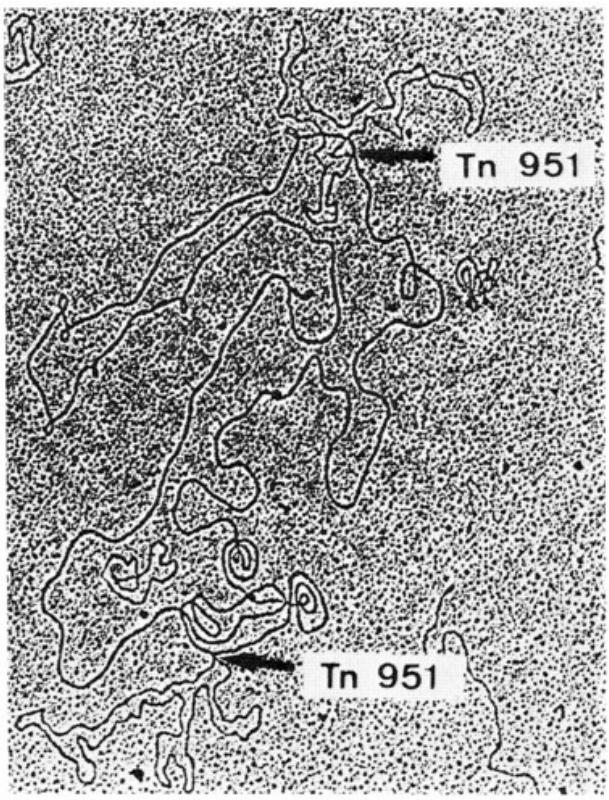

Fig. 2. Heteroduplex molecules formed between two different RP1 :: Tn951 molecules. The arrows indicate the positions where the single stranded loops (16.6 Kb) emerges from the 57 Kb long double stranded circular DNA

heteroduplex molecules will consist of a double stranded circular molecule having the length of the parent plasmid and two single stranded loops corresponding to the transposon (Fig. 2). The distance between the two insertion sites can be measured, but this type of analysis however doesn't allow to localize the insertion sites on the plasmid map, at least without any refinements.

This kind of information can be inferred from experiments using restriction endonucleases. These nucleases cleave DNA at very specific sites and thus generate defined fragments from a given replicon. These fragments are subsequently resolved by agarose gel electrophoresis. Partial digestions and double digestions using simultaneously two different nucleases allow to align these fragments and to draw a map, referred to as the physical map. Insertion of a transposon into a plasmid will either introduce new cleavage sites, generating new bands, or specifically increase the length of a given fragment. If one knows the physical map of the target plasmid, it is possible to map the transposition sites and even to determine the orientation of the transposon, making use of the restriction nucleases.

Electron microscopy and restriction nucleases have been used to map the insertion sites of various transposons on a number of targets. Clearly, the number of insertion sites is usually very high: we mapped eight insertions of Tn951 on RP1 and found eight different loci (Fig. 3). Heffron and co-workers (1975a) could detect at least 19 sites for Tn2 on an 8 Kb long target plasmid!

Considering that transposition consists of the insertion of DNA sequences of a certain length into a genome, it is not surprising that it sometimes leads to the inactivation of genes. Plasmid pGC530 is an RP1: Tn951 derivative where the insertion was found to map at coordinate 13 Kb, i.e. within the

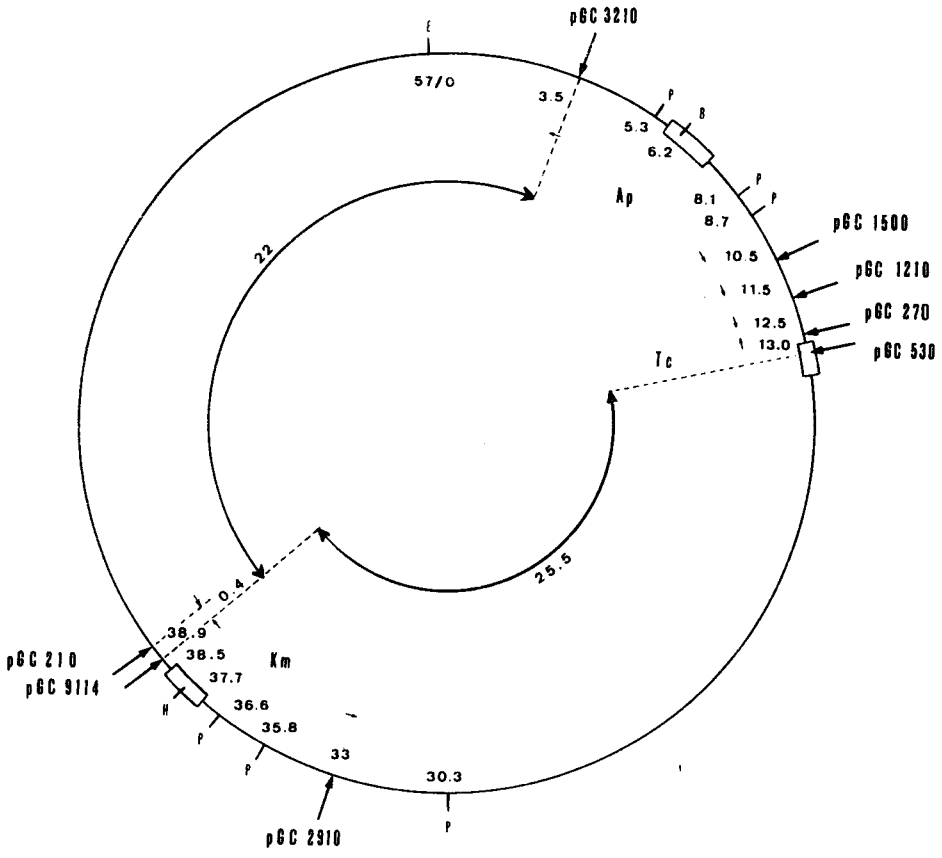

Fig. 3. Physical map of RP1 showing the 8 insertion sites of Tn951. pGC3210. pGC1500. pGC1210. pGC270. pGC530. pGC2910. pGC9114 and pGC210 are the 8 RP1 :: Tn951 derivatives. E = Eco R1 endonuclease cleavage site; B = Bam H1 endonuclease cleavage site; P = Pst 1 endonuclease cleavage site; H = Hind III endonuclease cleavage site; Ap = ampicillin resistance gene; Tc = tetracycline resistance gene; Km = kanamycin resistance gene. Coordinates are in kilobases. The inner lines refer to distances measured in heteroduplex analysis

tetracycline resistance gene (see Fig. 3). In accordance with our physical mapping, pGC530 does no more confer resistance to tetracycline. Insertion of a transposon not only abolishes the function of the gene into which the element lands but it can also be polar, affecting the expression of the genes located downstream on the transcriptional wave (Kleckner et al., 1975). For instance, Tn2 can land in the sulfonamide resistance gene of a plasmid and this inhibits the expression of the neighbouring streptomycin resistance gene.

Since transposons behave like mutagens and since their insertion can readily be physically mapped, they appear as valuable tools for the genetical analysis of plasmids. Tn7 has recently been used to analyse the transfer genes of the plasmid RP4 (Barth et al., 1978).

Transposons can sometimes be excised from a replicon where they have been inserted but the excision frequency is usually lower than the insertion frequency. Moreover, the process is often not precise. In our hands, a strain carrying pGC530 does not revert to tetracycline resistance, indicating that Tn951 does not excise accurately at a detectable frequency.

D. Structure of the Transposons

Five years ago, Sharp and co-workers (1973) discovered inverted repeats on plasmid DNA. These inverted repeats consist of identical DNA segments occuring twice in a genome but in opposite orientation. When a DNA fragment containing such inverted repeats or palindromes is submitted to heteroduplex analysis, intrastrand annealing of these fragments takes place giving a mushroom like structure (Fig. 4), consisting of a double stranded stem and a single stranded loop.

Later on, the mushroom like structure observed by Sharp et al. turned out to contain tetracycline resistance genes, in the loop. Moreover, the tetracycline transposon of Kleckner et al. (1975) gave the same structure in heteroduplex analysis. Other transposons were also found to consist of a DNA sequence flanked by two inverted repeats. For instance, Tn1 is flanked by a repeated sequence about 140 base pairs long (Rubens et al., 1976) and Tn5 is flanked by a repeated sequence ten times longer (Berg et al., 1975). These two inverted repeats do not correspond to any known Insertion Sequence. Tn9, on the other hand, is bordered by the well known sequence IS1 (Starlinger and Saedler, 1976) but repeated in tandem. Transposons are thus, often, if not always, flanked by repeated sequences.

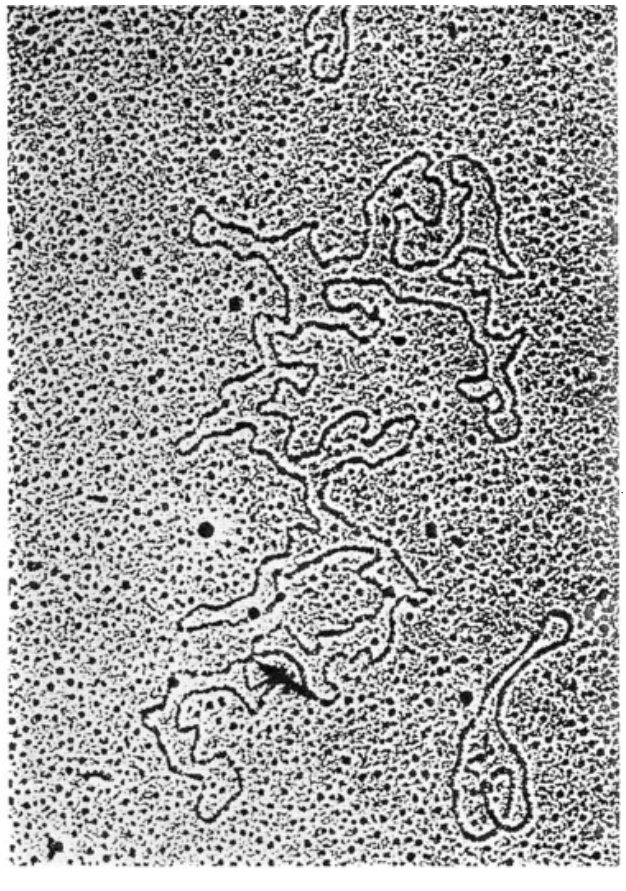

Fig. 4. Homoduplex formation of pGC1 DNA. The arrow indicates a small double stranded DNA region due to reannealing of small inverted repeats

E. Mechanism of Transposition

Although a number of models have been proposed, we have, so far, no clear understanding of the transposition mechanism. Experimental data from Heffron et al. (1977) suggest that a central region and the termini of Tn2 are required for transposition. The most attractive interpretation of their data is that the terminal sequence of the transposon serve a structural role while the central region encodes an enzyme required for transposition. Apart from that, it is not yet clear whether the process requires the recognition of a special sequence on the target.

F. Role of the Transposons in the Evolution of the Bacterial Plasmids

The discovery of transposable elements seems to account significantly for the structural and genetic diversity of plasmids. It has been known for long indeed that there is no relation between the genes carried by a plasmid and the type of plasmid. For instance, the TEM β-lactamase can be coded by plasmids that belong to, at least, 14 different incompatibility groups (Matthew and Hedges, 1976). It is very tempting to believe that the spread of Tn1 and Tn2 accounts for this situation. In accordance with this hypothesis, Heffron et al. (1975b) showed that, indeed, a great variety of different plasmids encoding the TEM lactamase contain a 4,5 Kb long sequence in common, while the TEM gene itself is much smaller.

The evolution of plasmids would thus essentially be due to terminus-site-specific recombination. Plasmids themselves seem to consist of two parts: one is essential for replication and transfer of the plasmid, while the other is a succession of transposons (and defective transposons?) acquired and lost during the history of the plasmid. In other words, plasmids would be nothing else than genetic carriers whose function is to replicate and propagate transposons.

G. Origin of the Transposons Carried Genes

So far, little is known about the origin of the transposons carried genes. For instance, the TEM β-lactamase coded by Tn1 and Tn2 appears different from all the known chromosomal β-lactamases.

For the lactose fermentation genes, however, we observed that the Tn951 coded operon is identical to the E. coli chromosomal lactose operon. In heteroduplex analysis, we detected 5,6 Kb homology and this length accounts for the genes encoding the repressor, the β-galactosidase and the permease (Fig. 5) (Cornelis et al., 1978). This indicates that, at least in some case, a transposon coded and a chromosomal (to date non transposable) operon may evolve from a common ancestor.

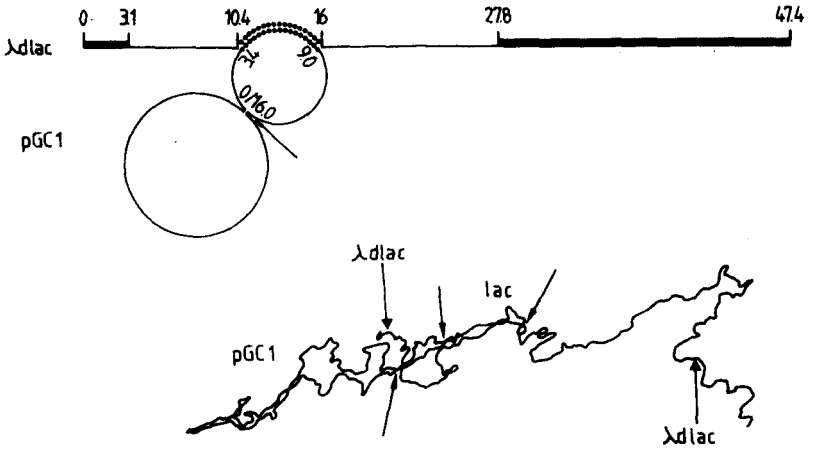

Fig. 5. Diagram of an heteroduplex molecule showing the 5.6 Kb homology between the E. coli chromosomal lactose operon (here carried by λ hdlac) and the Tn951 lac operon, here carried by pGC1

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