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Research Article

Antibiotic resistance crisis and revival of Beta Lactams old or new

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Abstract

In a recent article I have proven F is not a single replicon but co-integrate of two replicons – F and R but never allowed to function simultaneously by its transposon Tn 1000(gamma, delta). Antibiotic crisis begins in 1960 in a reputed Tokyo hospital by abuse or overuse of beta lactam. Therefore, their clinicians failed to cure a female patient who was admitted with her unbearable abdominal pain. Penicillin or its newer derivatives did not give her any relief. This patient keeps crying until her body immunity helps her to recover. Stool culture of this patient shows the presence of Shigella flexneri which is no longer sensitive to ampicillin.

Clinicians accepted the defeat of our antibiotics and therefore antibiotic resistance crisis begins. Their clinicians had no knowledge of transposons (mobile DNA elements). For their ignorance these transposons contaminated the Tokyo Hospital environment. In 1973 Dr. S N Cohen, Stanford University Medical Faculty (USA), started in-vitro gene cloning experiment using a cloning vector pBR322 which is really antibiotic resistant plasmid carrying two antibiotic resistance **transposonsTn4 and Tn10**. The transposon Tn 1000 of E.coli K-12 sex factor F is not the same but on the contrary the Tn 1000 reduces the copy number of pBR322 .

Keywords: F plasmid Replicons, Transposon Tn1000 controls replicons, Antibiotic resistance transposons(Tn 4,Tn 10), used in-vitro gene cloning

Introduction

In 1960 antibiotic resistance crisis was born in a reputed hospital of Tokyo by abuse and overuse of ampicillin. Their clinicians concluded that the antibiotic resistance is real because the pathogen Shigella flexneri isolated from the stool culture of a female patient admitted into their reputed hospital in Tokyo is no longer sensitive to ampicillin.

As illustrated in Fig.1, F plasmid contains three replicons F1A, F1B, F1C. The F1B is not a complete replicon but it may have other functions. F plasmid also carries three mobile DNA elements-IS2, IS3 and Tn1000 [1]. These mobile DNA elements are involved in the formation of three ***E.coli*** K-12 males, F+, Hfr and the F-primes. The Hfr males are formed by homologous recombination between IS2 and IS3 on F as well as on E.coli K-12 chromosome are stable like Hayes Hfr but the Hfr formed by the Tn 1000 is unstable [2]. In 1969 Palchaudhuri and Iyer have demonstrated how the male specific phage M13 cures the F plasmid which prevails as an extra -chromosome but in the stable Hfrs where F prevails passively being integrated into the host chromosome but in both males the phage M13 multiplies and releases by puncturing the cell wall of E. coli K-12 [3]. In a recent article I have proven F plasmid is not a single replicon but co-integrate of two replicons – F and R but never allowed to function simultaneously by the transposon Tn 1000 (gamma, delta) [1]. However, two F plasmids or two F prime plasmids are incompatible because F1A carries inc loci [3]. In fact, F1C is the replicon of R plasmid and does not revert back to F plasmid. In addition to these two replicons F1B is not a complete replicon but it may have other roles (Palchaudhuri S. unpublished data). Insertion sequences IS2 and IS3 are used in the formation of stable Hfr males without such knowledge of molecular biology there was a bitter conflict between Lederberg and Hayes (an army doctor in British India) for a decade.

Tn 1000 is also the transposon but unlike antibiotic resistance transposons Tn4 and Tn10, Tn 1000 appears to be involved in keeping their replicons in latent phase or in a monocopy state [3,4]. Fig 1. Shows the map of F plasmid with three replicons F1A, F1B, F1C and three mobile DNA elements IS2, IS3 and Tn1000.

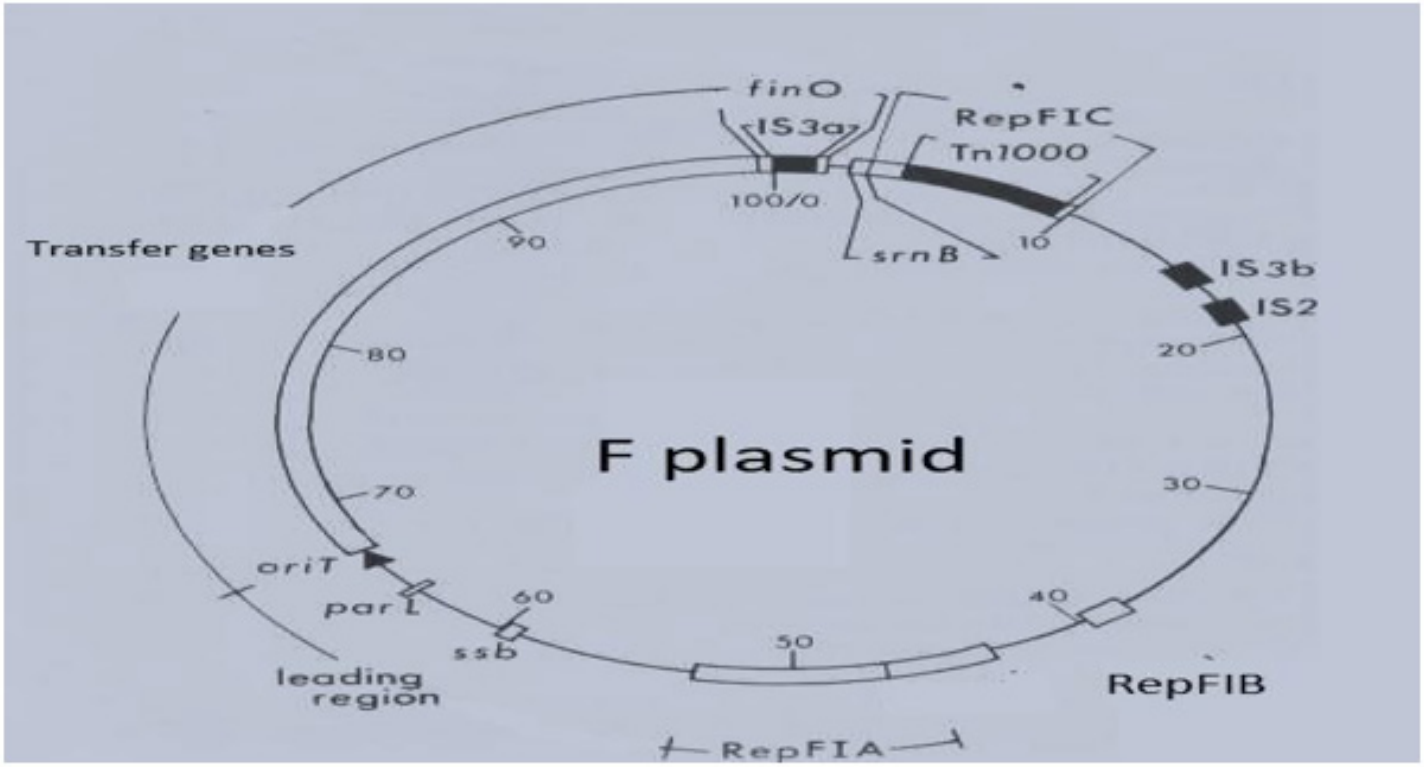

Figure.1. Map of F plasmid showing the location of three replicons F1A, F1B, F1C, its mobile DNA elements IS2,IS3,transposonTn1000 and srnB (inducible modifier of host membrane).

Method

EM / heteroduplex was formed between F plasmid and R plasmid following the procedure of Sharp et al but later modified by Palchaudhuri et al [5-7].

Results/Discussion

F1A with inc loci is apparently responsible for the incompatibility between two F plasmids or two F prime plasmids. Therefore, two replicons are never allowed to function simultaneously but their functional states are stringently controlled by the transposon Tn 1000. Besides these two replicons we have previously accepted as if the Ent plasmid P307 is another new replicon but now we want to make it clear **that the Ent plasmid is not any new replicon but** it is really the deletion derivative of the antibiotic resistance plasmid pCG86 [5-8].

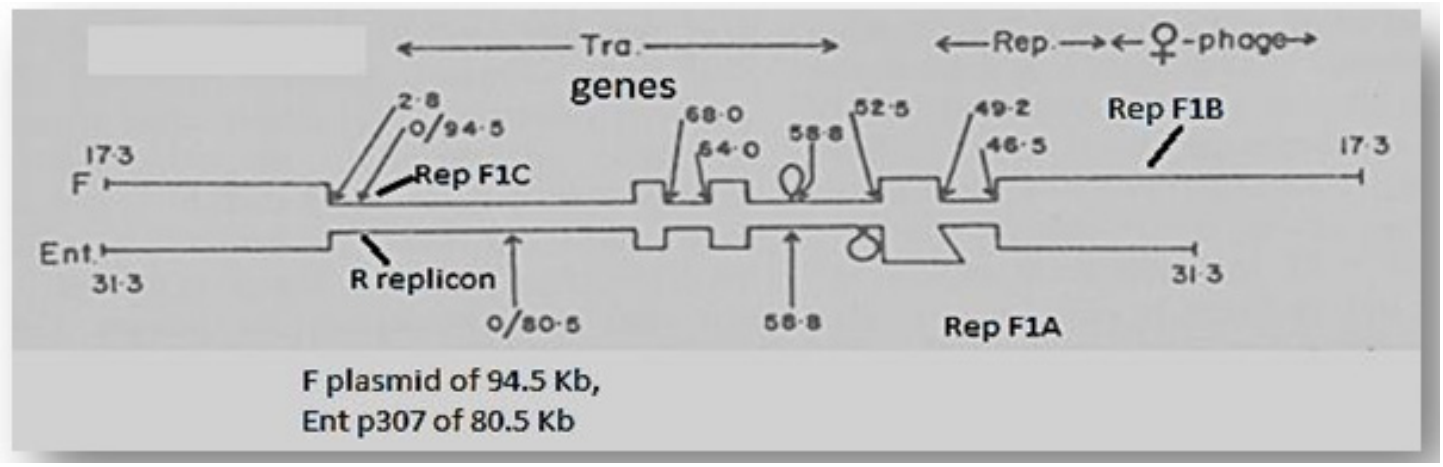

Figure 2. Heteroduplex formed between Fins(an insertion at 58.8F) and Ent P307 (the deletion mutant of R plasmid pCG86).

Curiously, Ent P307 does not show incompatibility with F plasmid because this Ent P307 is the deletion derivative of R plasmid pCG86 with replicon F1C which is compatible with F1A with inc loci. In 1975 we have characterized the plasmid Ent p307 which was evolved as a deletion derivative of the pCG86 with R replicon (**RepF1C**) [4].

Evidently the Ent p307 is the deletion derivative of pCG86 but carrying the enterotoxin genes (both heat stable and heat unstable). We must not forget that Carlton Gyles sent the same ETEC with pCG86 also to his mentor Professor S.

Falkow who saved it in an agar stab but without any antibiotics [8,9]. It became antibiotic sensitive being in his laboratory or when I grew this strain in nutrient broth and tested its sensitivity to ampicillin or tetracycline (Palchaudhuri S, unpublished data).

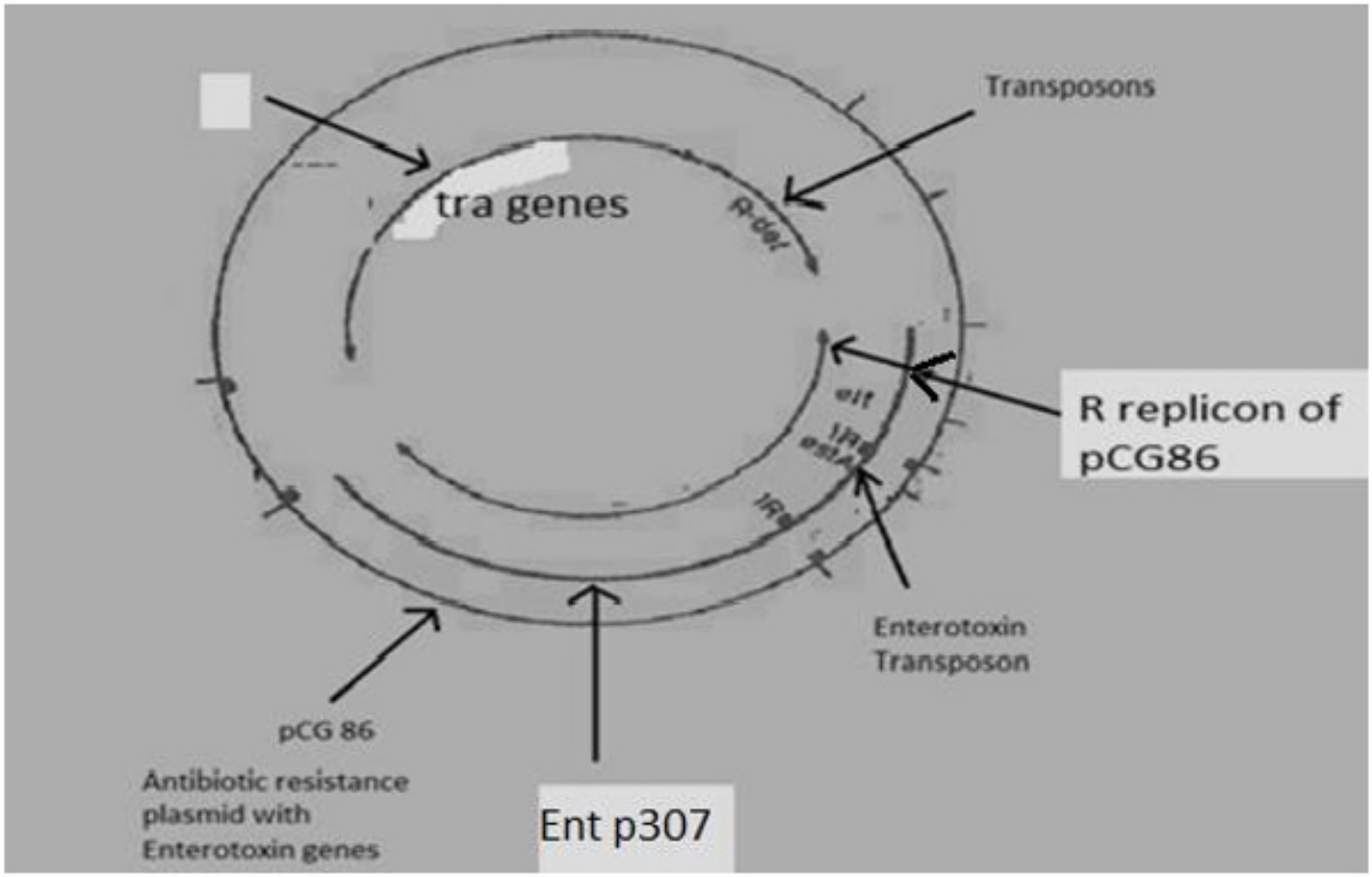

Figure 3. Replicon homology between antibiotic resistance plasmid pCG86 and Ent P307

In the year 1977 Gyles, Palchaudhuri and Maas have published an article in **SCIENCE (1977**) showing the antibiotic resistant plasmid pCG86 also carries genes for the production of enterotoxin (both heat stable and heat labile) [8-10].

Since most ***ETEC*** isolates from the stools of piglets carry antibiotic resistance genes as well as genes for enterotoxin production. In fact, we want to make it clear that **there is no enterotoxin replicon** but Ent plasmid P307 carries genes for the production of enterotoxin and also lacking a few tra-genes. What is more, F is never a single replicon but co-integrate of two replicons – F and R. From self-annealing experiments of Mazaitis et al, they found that the plasmid pCG86 contains two DNA segments bounded by inverted repeats suggesting the presence of two transposons [11,12]. One of these segments carries the gene for tetracycline resistance and the other carries the gene for heat stable enterotoxin production. We also found that about half of pCG86 is completely homologous with Ent P307 [5].

In 1973 Dr Stanley Cohen had a brief telephone conversation with Professor WK Maas and asked him about the location of R plasmid replicon. Later on, I had a long conversation with Professor Maas on why Stanley Cohen wanted to know the replication origin of antibiotic resistance plasmid (personal communication). Regardless Professor Maas and I were impressed by their strength of EM heteroduplex technique as used by Sharp et.al (1973) but my problem was how I can learn such a technique [6]! In 1974 Professor Maas talked with Professor N Davidson who agreed to send one of his associates to NYU school of Medicine to learn this technique by hands on experience [12-14]. An heteroduplex molecule formed between the F and Ent P307 shows two insertion loops; almost of the same size but located at two different distances of F plasmid

(Fig.2). After a year I was happy to confirm that the second insertion loop formed in this heteroduplex molecule, due to heat stable enterotoxin in Ent P307 but this loop was previously ignored. EM-heteroduplex analysis shows DNA sequence relationship between the two DNA bio-macromolecules, F plasmid and the antibiotic resistance plasmid R [1]. Sharp et al remained silent about the location of R replicon even in the absence of replicon F1A and its but showed the homology with RepF1C [8]. The transposon Tn1000 was still present but allowed the Rep F1C to function stringently in a monocopy state when Rep F1A was absent. I have just discovered that the antibiotic resistance transposons are immediately lost in the absence of their selection pressure. The plasmid pCG86 was evolved in ETEC pathogen, responsible not only for the piglet's diarrheal disease in Canada but also allowed it to survive in the presence of antibiotics abused in veterinary medicine.

Doses of drugs and duration of treatment varied depending on the recovery of pigs. Therefore, I was excited to observe the loss of antibiotic resistance characters (transposons) when the R plasmid pCG86 was transferred into our laboratory bacterium E. coli K-12(711, Falkow) and stored in an agar medium (stab) but without any antibiotics. The antibiotic resistance transposons are selectively lost when its growth environment completely free from antibiotics. Evidently Dr. Carlton Gyles sent the same bacterial strain ETEC pCG 86 carrying plasmid to his mentor Professor S. Falkow stored it in an agar stab at room temperature in 1975. Professor Maas received the same **stab(P307)** from Professor Falkow in the same year. The Ent p307 is carrying genes necessary for the production of enterotoxin (both heat labile and heat stable) but its replicon is R replicon and originated in an E. coli K- 12 derivative 711 after its transfer and storage in the agar stab without any antibiotics.

I spent few years with Professor W.K. Maas at NYU school of Medicine as his Research associate but he was always thinking to classify all these plasmids of Gram- negative bacteria by their incompatibility but the confusion arises when I discovered that the plasmid Ent P307 is compatible with F plasmid (S. Palchaudhuri, unpublished data)! We have described an unusual conjugative plasmid pCG86 present in ETEC, isolated from a piglet with diarrhoea. This plasmid pCG86 carries genes for heat-labile enterotoxin and heat- stable enterotoxin and also carries genes for resistance to tetracycline (Tc), streptomycin (Sm), sulphonamides (Su)and mercury (Hg). Now these antibiotic resistance characters are recognised as transposons. **Interestingly heat stable enterotoxin is also a transposon. Fortunately, the transposon Tn1000 (gamma delta) of length 5.7Kb plays an extremely important role in the genesis of R plasmid from F plasmid but irreversibly.** In 1960, the presence of such an R factor in the pathogen Shigella flexneri, (close kin of E. coli K-12) begins antibiotic resistance crisis but abuse of in-vitro gene cloning technique makes it global.

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