

Mobile DNA Elements in *Shigella flexneri* and Emergence of Antibiotic Resistance Crisis

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Introduction

Prokaryotic mobile DNA elements were initially observed by Nobel Prize Winner Professor Barbara McClintock in 1983. Both IS and Tn elements are defined as mobile DNA elements capable of inserting into many different sites of a single chromosome or different chromosomes as discrete, non-permuted DNA segments [1,2]. In 1952 Dr Joshua Lederberg (a noble prize winner of 1958) has shown the presence of such elements IS1, IS2, IS3 and Tn element, Tn1000 or gamma delta in the first quadrant of fertility factor F in *E. coli* K-12 (Figure 1) [3].

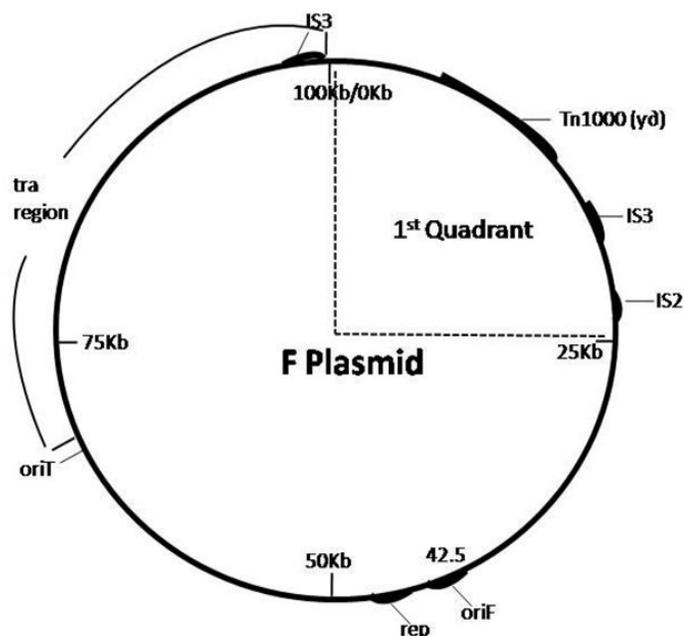


Figure 1: F Plasmid-Lederberg's Fertility factor F of *E. coli* K-12 [4].

F Plasmid-Lederberg's Fertility factor F of *E. coli* K-12.

- 100 Kb.
- ori F-origin of F plasmid replication. (With the sequence for dna A gene to function)
- rep-replication functions.
- IS-insertion sequence (IS2, IS3 and IS1 copies present in the host *E. coli* K-12.
- Tn-Transposable elements (gamma-delta, acts both as IS and Tn elements)

It remains to be understood how such F plasmid was evolved and it seems that the mobile DNA elements have played a role [5]. *Shigella flexneri* is closely related genetically, both are Gram negative. *E. coli* and *S. flexneri* produce F pili which is essential for bacterial conjugation. Extensive research work has revealed that the mobile DNA elements play a central role in inducing random mutations (point, deletions, so on) and thus create a crisis of antibiotic therapy.

Tokyo Hospital and Origin of Antibiotic Resistant Plasmid

In 1960, Beta-lactams could not effectively kill the pathogen *S. flexneri*, therefore patients keep suffering from Shigellosis. Extensive research work showed the presence of *Shigella* in the stool of a Japanese rich female patient in a reputed Tokyo hospital [6]. Prof. T. Watanabe (1960), after 3-4 months of extensive academic research, has shown the R factor, especially R6-5 is self-transmissible between *E. coli* K-12 and *S. flexneri* [7,8]. R factor appears to be an extra-chromosomal plasmid and is capable of showing gene transfer from *S. flexneri* to *E. coli* K-12 or vice versa

via conjugation. Antibiotic resistant character has been identified as R plasmid, an extra-chromosomal element that was present in the antibiotic resistant *S. flexneri* [9]. Several self-transmissible R plasmids present in epidemic strain of *Shigella* in 1960, R1, R6, R100, have been detected. R6-5 isolated in 1972 during antibiotic resistance crisis from *Shigella* was characterized [7]. We are afraid that such self-transmissible plasmid (R6-5) has been extensively used by Nobel Prize winner Prof. Stanley N. Cohen, in all his *in vitro* gene cloning experiments 1972-onwards [10]. Question arises, where does such R plasmid come from? We think, F plasmid (1952) of Dr. Lederberg's lab has played a role in the evolution of R plasmid in *Shigella* by the selection pressure of antibiotics as used and overused in Tokyo hospitals, ignoring Dr. Alexander Fleming's repeated requests to stop abuse of antibiotics from 1951 [11].

Self-transmissible F and R plasmid

F and R are self-transmissible plasmids. F plasmid does not possess any antibiotic resistant character, but carries IS and Tn elements. The $\gamma\delta$ sequence has been recognised both as IS and Tn element, with an ability to cause spontaneous deletions. How do these plasmids correlate? Both plasmids replicate stringently (1-2 copies per host chromosome) and are self-transmissible by cell-to-cell contact (conjugation).

In 1952, Dr. J. Lederberg et al. have detected a fertility factor F in the course of his research work at in Rockefeller University, in New York City [3]. He has recognized that the F plasmid is present in *E. coli* K-12 as fertility factor, but apparently without giving any information about the source of F. Of course, he has observed in his laboratory that *E. coli* K-12 donor and *E. coli* K-12 recipient are different based on the presence or absence of F plasmid respectively [3]. In 1960, Dr. William Hayes has isolated a similar fertility factor from *E. coli* K-12 but in an integrated state and named it as HfrH [12]. That means it is capable of mating with a competent recipient to produce recombinants (gene transfer by conjugation) and it has been very useful in the development of bacterial genetics, more specifically *E. coli* K-12 genetics.

Major question: has there been any interaction between *E. coli* K-12 as donors and *Shigella* as recipients? We assume such interaction is responsible for giving rise to R plasmid in *Shigella* under the selection pressure of antibiotics. F plasmid is highly stable, but R plasmid shows dissociation into two components RTF and r-determinants (r-det) depending on the growth media [13,14]. A molecular comparison between the two plasmids shows that RTF has extensive DNA sequence homology with F plasmid. RTF and F are capable of autonomous replication and are self-transmissible by cell-to-cell contact. In *E. coli* K-12, are these two plasmids, R and F, stable or unstable? In previous publications, co-existence of F and R has not been reported [15]. However, based on available evidence we believe that **F plasmid has become R plasmid via RTF**. Self-transmissible F plasmid is equivalent to

RTF, both are replicons and they carry transfer genes essential for gene-transfer by conjugation. Still they are not identical in DNA sequences in their ori regions nor their biological functions like interaction of dnaA protein with ori [16-18]. Interestingly, these transfer genes form large operons encoding for production of F-pili which apparently plays a role in gene-transfer during conjugation and male specific phage M13 or R 17 adherence, penetration of genome and their subsequent multiplication [19].

Conclusion

Antibiotic resistant plasmid R6-5, originally isolated in 1960 epidemic of Tokyo, dissociates into two components: RTF and r-det. Until recently, r-det component has been wrongly interpreted. R-det actually are **antibiotic resistant transposons**. We conclude that F plasmid has become RTF component of R plasmid by combining or associating with a garland of transposons (r-det) via duplication of insertion sequence IS1 in a direct order. Garland of transposons has been elaborated in our next article. We must remember that transposons are mobile DNA elements that play a detrimental role in causing several incurable diseases from **pneumonia to cancer**. Mobile DNA elements are capable of inserting at random into the chromosomes of bacteria or humans depending on the physiological conditions of the host. However, all these r-determinants were previously assumed to have risen by the dissociation of R plasmids. **In fact these r-determinants are all transposons and not merely r-determinants**. We have until now characterized the r-det component as if it carries all antibiotic resistant transposons (ampicillin Tn1, Tn2 or Tn3, sulphanamide & streptomycin Tn4, tetracycline Tn10, kanamycin Tn5, mercury) [5,20]. Unfortunately, many reputed investigators have not made it clear how antibiotic resistant determinants become transposons. Modern medicine is victimised because of our indifference towards recognition of transposons. In 1960 during antibiotic resistance crisis in Tokyo, the transposons are born in the pathogen *Shigella flexneri*. We should accept that all antibiotic resistant determinants are transposons although it has not been recognised that way because previous investigators' have failed to define the real difference between antibiotic resistant determinants and their transposons, which have stunted the growth of the science until 1973. However, we must not forget that the antibiotic resistant determinants and transposons have different meanings [1]. What is more, Dr. Barbara McClintock (1983 Nobel Prize winner) has reported mobile genetic elements long time back in the course of her work on corn color.

Another Nobel Prize winner (1986) Professor S N Cohen has been using self-transmissible plasmid (R6-5) extensively in many of his *in vitro* gene cloning experiments till-date [10]. Until 2013, he has remained silent about the abuse of transposons even in his recent article of Proc. Natl. Acad. Sci. USA [10]. R6-5 contains several antibiotic resistant transposons, but unfortunately our ignorance is responsible for such abuse. Is *in vitro* gene cloning

experiments with transposons responsible for the increased occurrence of cancer regardless of age [21]?

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