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Research Article

Gamma-Delta or Transposon Tn1000 forms Unstable F-Prime Plasmid KLF-5 in the Proximity of Oric

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Abstract

The F prime plasmid KLF5 consists of two components, one component is F plasmid (100Kb) and the other component is chromosomal segment of length 125Kb containing metBJF and argECBH operons, is unusually unstable. The parent of this KLF5 is an Hfr donor Ra-2, an *E.coli* K-12 derivative in which F is integrated into the chromosome at a site, probably gamma delta which is proximal to the oriC. Such an integration of F occurs by a cross-over between gamma delta of F and the copy of gamma delta prevailing in the chromosome (sfa site). Duplication of gamma delta (Tn1000), one copy at each junction of the F and the *E.coli* K12 chromosome. The Hfr Ra-2 thus formed is unstable. F factor becomes RTF first by the self- deletion effect of gamma –delta (2.8F -8.5F) to transpose into the F oriV at 42.5F. In such genetic event the RTF (replicon) alters few bases and then associates with available transposons via IS1 (duplicated in a direct order). These transposons are not just antibiotic resistance determinants of R plasmid but transposons which can translocate into any site on the same chromosome (spontaneous mutations)! Such an R plasmid confers on its host bacterium *Shigella flexneri* an ability to survive in penicillin or ampicillin. We dare now to predict that F replicon has become R replicon (RTF) by a subtle change in the oriV region of Dr Lederberg's F plasmid.

Keywords

F plasmid; E.coli; Plasmid; Oric

Introduction

Our article published in MGG in 1976 has shown that the F prime plasmid KLF5 consists of two components, one component is F plasmid (100Kb) and the other component is chromosomal segment of length 125Kb containing metBJF and argECBH operons, is unusually unstable [1]. The parent of this KLF5 is an Hfr donor Ra-2, an *E.coli* K-12 derivative in which F is integrated into the chromosome at a site, probably gamma delta which is proximal to the oriC. Such an integration of F occurs by a cross-over between gamma delta of F and the copy of gamma delta prevailing in the chromosome (sfa site). Duplication of gamma delta (Tn1000), one copy at each junction of the F and the *E.coli* K12 chromosome. The Hfr Ra-2 thus formed is unstable. We speculate that the F-prime plasmid KLF5 if formed by such Hfr donor by type II excision then the instability of the F-prime plasmid KLF5 is not any surprise. The chromosomal segment flanked by the gamma-delta in a direct order satisfies the definition of a transposon [2-4]. Furthermore, we want to demonstrate that the Tn1000 containing the chromosomal segment of KLF5 (approx.125 Kb in length) is translocate into the stable F'trp (F155) when they are forced to co-exist in the same RecA *E.coli* K-12 host and by the selection pressure. F'trp or F155 plasmid has been previously characterized (1). This is formed by type I excision replacing the F sequence 8.5F - 16.3F by a chromosomal sequence containing att80 (bacteriophage phi80 attachment site) and trp operon dissociates into F and its chromosomal component met+arg+ even in a Rec A strain. Transposition of this chromosomal component is triggered by the selection pressure apparently created by introducing a stable type I plasmid segment of F155 into the recipient carrying KLF5, which occurs by selection for operons met, arg and trp present in two different F primes with an assumption that they are present on two autonomously replicating F-prime plasmids [5]. The plasmid F'trp+ or F155 is quite stable even in RecA strain but the KLF5 is unstable and dissociates into two components: F and chromosomal segment, they are held together by the gamma delta similar to that of IS1 in the self-transmissible antibiotic resistance plasmid R6-5 [6]. We wonder if F becomes RTF in *E.coli* K-12 or *S.flexneri* by the translocation of Tn1000 apparently induced by the selection pressure of antibiotics (intramolecular transposition). Tn1000 or gamma delta moves by the replicative mechanism [7,8].

In 1973 Dr SN Cohen has initiated in-vitro gene cloning technology with a multicopy replicon of colicin ColE1 ligated to an antibiotic resistance transposon of antibiotic resistant plasmid R6-5 without the prior knowledge that R6-5 carries apparently no antibiotic resistant determinants but transposons (eg- amp^r Tn 1, Tn2 or Tn3). Briefly, the R6-5 consists of two components: RTF and a garland of transposons (for many years they are referred to as r-determinants!). These two components are linked together by the IS1 sequence repeated in a direct order [6,7]. Significantly, in F-prime plasmid KLF5 the chromosomal segment containing operons metBJF, ppc and argECBH is similarly flanked by the gamma delta sequence in a direct order? Wang et al have demonstrated the inversions and deletions are generated by a mini gamma delta transposon [8]. Unfortunately these investigators have used multi-copy cloning vector pBR322 already carrying transposon to understand the gamma delta! What is worse, our enemy Tn1000 got an opportunity to escape. Transposon Tn1000 is a member of the Tn3 family [2]. We think that the abuse of antibiotics has activated F's Tn1000 (2.8F -8.5F) to alter the oriV at 42.5F by gradual deletions from 8.5F until it becomes RTF and transfers itself into *S.flexneri* forms R plasmid using IS1 to tie with the garland of trasposons available to grow in antibiotics. In our separate article, we will present a road map to stop the spread of Gamma delta or Tn1000 (maxi or mini) which is capable of changing the F plasmid into an R plasmid [9].

Materials and Methods

Bacterial strains

The relevant genotypes of the strains used are listed below, and the location of the genetic markers.

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F' trp/7: F' trp$^+$ /trp argH thr leu thi lac recA str

KL5/7: F' met$^+$ ppc$^+$ arg$^+$ /trp argH thr leu thi lac recA str

7F$^-$: trp argH thr leu thi lac recA str

F' met$^+$ arg$^+$ trp$^+$ /trp argH thr leu thi lac recA str

7F$^+$: F/trp argH thr leu thi lac recA str

K12 (lambda) F$^+$ (Lederberg)

Arg denotes the cluster of genes argE argC argB argH; met denotes the cluster metB metJ metF; trp denotes the tryptophan operon. Details of the construction of the fused, F'-carrying strains have been previously reported [1].

Electron microscopy and measurements

Super twisted molecules are converted to open circular forms by x-ray doses ranging from 200-750 rads given at room temperature, higher molecular weight DNA receiving smaller doses than lower molecular weight DNA. DNA in 0.01 M phosphate buffer-1 mM EDTA (pH 7.2) is diluted to about 2 µg/ml in 0.8 M ammonium acetate (pH 5.0), and cytochrome c is added to a final concentration of 100 µg/ml. An aliquot (0.1 ml) of this solution is spread over a hypophase of 0.3 M ammonium acetate (pH 5.0). The DNA-cytochrome c films are picked up on thin carbon film supports, deposited on a layer of parlodion mounted on Pt grids (Siemens type) and stained with 0.1 mM uranyl acetate in acetone. The electron microscope (Siemens 101 Elmiskop) used for the preparation is calibrated for a set of exposures at the same operational magnification by a grating replica (2160lines/mm, Fullam, Inc.). The dark field mode used in these studies operated on the magnetic tilt with short exposure times (2 sec).The negatives are enlarged eight times by a rear projector, and the DNA is traced by a hand-held stylus on a RAND tablet (Grafacon 1O1OA as a screen). The contour lengths (total or segments) are computed with a PDP-8 computer (Digital Equipment Corp.) and give slightly larger lengths (+4%) than those traced by a map ruler at the same modifications.

Results

Difference between Type I and Type II F' plasmids

Previously we have shown that two F-prime plasmids when introduced into the same bacterial cell, they form a single F-prime plasmids or they integrate into the host chromosome [10]. Figure 1 shows the location of IS and Tn elements (gamma-delta). Type I F' is formed by homologous recombination between IS2 and IS3 present on F and same sequence on *E.coli* K-12 chromosome. Since formation of fused F' factors can occur in recA strains, it must either be mediated by an F specific recombination system, for whose existence there is so far no evidence, or it must depend on processes that are independent of the bacterial homologous recombination system. By 1972, we have accepted that the *E.coli* K-12 donors exist in three different physical states: F+, Hfr and F primes [11]. F+ donor of *E.coli* K-12 becomes Hfr donor by the integration of F at different sfa (sex-factor affinity) sites of the host chromosome. We assume that these sfa sites are representing the insertion sequence (IS) like IS1, IS2, IS3, gamma-delta (γ∂) etc. In Hfr donor Ra-2, F plasmid is not highly stable therefore occasionally there is an aberrant excision of F-plasmid. Such excision gives birth to F-prime donors KLF-5 carrying a segment of chromosome with arg ECBH and met BJF operons. In this work we have analysed two F-primes one is type I and the other one is type II. We have taken into consideration that gamma-delta sequence makes the type II F-prime plasmid unstable whereas type I used in this study is always stable. Type I plasmid, F-prime trp+ (F-155) has already been well characterized by EM Heteroduplex analysis, therefore it is not discussed in details [1]. The type II F-prime plasmid is KLF5 (argECBH+, metBJF+): is very unstable and dissociates into two components one is complete F-plasmid and the other component containing a few operons (arg+ met+).

Type II F-prime (KLF5) is highly unstable

Self-hybridization of Type II KLF-5 plasmid DNA as shown in Figure 2a, 2b supports that KLF-5 dissociates into two components: complete F (100 Kb)and chromosomal segment carrying metBJF, ppc and argECBH (125 Kb). We have observed heteroduplex molecules formed between KLF-5 and F plasmid which is a segregant of complete KLF-5 (Figure 2b). Double stranded DNA formed is 100 kb long and the remaining 120 kb is single stranded DNA. The double stranded DNA region represents the homology between KLF-5 and the F-plasmid. The single stranded DNA region contains arg$^+$ met$^+$ chromosomal segments (120 kb). F and F_{ins} are used as reference molecules. Coordinates on F sequences are expressed in Kb units followed by suffix F and coordinates on chromosomal genes are also in kb units but followed by suffix B.

The F-prime plasmid was isolated from an unstable Hfr strain Ra-2 donor by mating with a *recA* recipient and selecting for a marker gene which enters early [4,12]. KLF5 does not contain any distal markers indicating KLF-5 is a combination of F-plasmid and gamma-delta transposon (Tn1000). We must not forget the location of oriC (an origin *E.coli* K-12 chromosome) is proximal to KLF5. How is this unstable KLF5 formed from its Hfr parent? It is already known that Ra-2 is unstable Hfr strain. Integrated F-plasmid is flanked by gamma-delta and therefore we think gamma-delta is responsible for such instability. The Hfr (Ra-2) chromosome formed in which gamma delta is located at the F-chromosome junctions appear to be quite unstable. We think this is a transposon Tn1000 carrying metBargECBH segment of the chromosome and dissociates into two components like that of antibiotic resistance plasmid R6-5 [13].

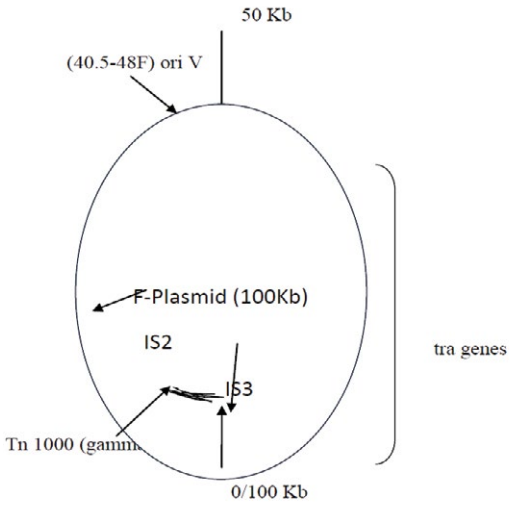

Figure 1: Physical map of F-plasmid showing the location of IS elements, Tn element Tn1000, ori V (origin for F-plasmid replication) and tra genes (absolutely necessary for conjugal transfer). Tn1000 plays duel role, both as IS sequence and as Tn element (transposon).

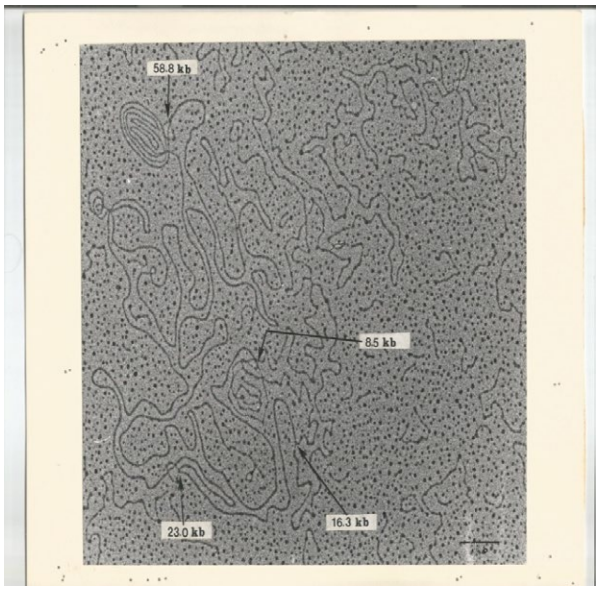

Figure 2(a): Electron micrograph of a heteroduplex molecule formed between these F and F'plasmid (KLF5). Two single stranded deletion loops of length 1.4Kb are located at 23.0F and 1.4Kb at 58.8F. The reference F_{ins} molecule contains these two loops.

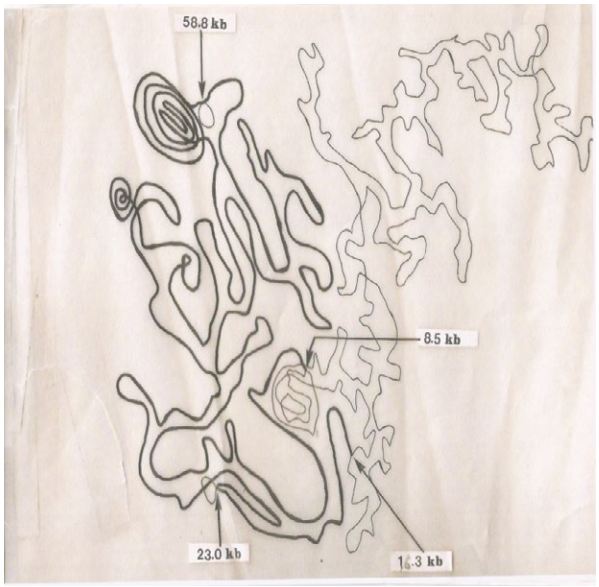

Figure 2(b): An heteroduplex molecule formed between F and F'plasmid (KLF5) carryng several chromosomal genes flanked by the gamma delta elements in a direct order. A tracing of an original electron micrograph is shown.

On the basis of data available, we must be careful about those unstable F-primes knowing that KLF5 is formed by a type II excision with junctions between F and its host chromosome. It is also a possibility that the type II excision does not depend on bacterial conjugation. KLF5 is highly unstable and it dissociates into two components even in recA strain: the components are F-plasmid by itself and the other component is chromosomal segment (arg+ met+) flanked by gamma-delta and carries no replicon. In the course of its growth in a rich broth, the population is carrying only F plasmid (segregants) and lacking chromosomal markers. Significantly all of them have retained the sensitivity to male specific phages R17 and M13, thus confirming the presence of F-plasmid. Obviously, F-plasmid is retained stably by the segregants of the original host *E.coli* K12 but all chromosomal markers are transposed via gamma-delta. Therefore gamma-delta does not require any replicon to transpose but it carries a large chromosomal fragment (arg+ metB+) to jump onto another replicon.

Ø80dmet and Ø80darg are transducing bacteriophage particles have been constructed and physically and genetically verified [14]. This data supports that the genome of these transducing phage particles carries gamma delta transposons and because of this viral life gamma-delta will spread widely.

Discussion

In 1952, Dr Joshua Lederberg has identified the presence of F plasmid as a bacterial fertility factor in *E.coli* K-12 [15]. In subsequent years, this F -plasmid has played an important role in developing *E.coli* K12 genetic linkage map [16]. However, in 1972 we have published an article that demonstrates how the IS (IS2, IS3) and gamma-delta (Tn1000) elements in a single quadrant of the *E.coli* -F plasmid (Figure 1) plays a role in changing if autonomous state of existence, integrates into the host chromosome and forms an Hfr donor. Then F becomes F-prime by its aberrant excision from the Hfr donor together with the chromosomal DNA segment, adjacent to the F and chromosomal junctions. When such excision covers just one junction then it is called type I excision. However, type II excision covers both the junctions and the F' formed are often unstable (thus leads to formation of transposons). We have neither seen the co-existence of two autonomous F-primes nor the coexistence of F-prime in the Hfr donor (incompatibility).

Like IS1 element gamma-delta can also delete itself. In support of this statement we want to remember why all the *E.coli* K-12 strains do not have gamma-delta, not only gamma-delta but also copy number may vary by self-deletion. Such a genetic behaviour is common to both IS1 and gamma-delta. Unlike ColE1 plasmid, copy number of R and F is stringently controlled by the host bacterium. When F and R plasmids are introduced into the same bacterial strain (*E.coli* K-12) they co-exist leading us to accept that there is a subtle difference between these two replicons. We speculate that the transposon Tn1000 introduces such difference. The region coding for a putative repA1 protein is interrupted by the transposon Tn1000 and shows no homology with the repA1 region of RepFIIA replicon [17]. They may compete for a site in the membrane to attach or for the supplies required for biosynthesis. These supplies include DNA polymerase I, II, III. In 1952, Dr Lederberg has reported a fertility factor F which is responsible for bacterial conjugation by cell to cell contact (15). In 1960, R-plasmid has been detected in Tokyo Hospital, based on their observation that Gram negative *Shigella* is no more sensitive to ampicillin. This helps us to speculate that F factor becomes RTF first by the self- deletion effect of gamma –delta (2.8F -8.5F) to transpose into the F oriV at 42.5F. In such genetic event the RTF (replicon) alters few bases and then associates with available transposons via IS1, an insertion sequence (duplicated in a direct order). These transposons are not just antibiotic resistance determinants of R plasmid but transposons which can translocate into any site on the same chromosome (spontaneous mutations)! Such an R plasmid confers on its host bacterium *Shigella flexneri* an ability to survive in penicillin or ampicillin. This is the beginning of antibiotic resistance

crisis in Tokyo hospital (Japan, 1960) and best index of treatment-failure induced by the overuse of antibiotics and probably by their physicians. Based on the nucleotide sequence analysis of Saadi et al we dare now to predict that F replicon has become R replicon (RTF) by a subtle change in the oriV region of Dr Lederberg's F plasmid [17,18].

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