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Research Article

MOBILE DNA ELEMENT Tn1000 IN INSTABILITY OF *E.coli* K-12 CHROMOSOME

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ABSTRACT

Duplication of Tn1000 or gamma-delta begins with the integration of F plasmid via gamma delta sequence into the chromosome of E.coli chromosome forms an unstable Hfr donor(Ra-2). F prime KLF5 (met B+argE+) isolated from such a donor (Ra-2) is equally unstable. When grown in a rich broth KLF5 dissociates into two components, F plasmid and the chromosomal segment. When such an unstable F-prime (KLF5) is introduced into another recA E.coli K-12 recipient already carrying a stable F-prime trp+, the stable recombinant F-prime carrying genetic markers trp +arg+met+ from both F-primes, is formed. Complete physical analysis of such recombinant F-prime plasmid shows the translocation of gamma-delta to a site proximal to phi80att site of F-prime trp plasmid. F component of KLF5 is lost and the lacXYZ operon of F'trp is deleted out! . F plasmid of KLF5 is lost. After these genetic events, the F-prime trp+ plasmid is still temperature sensitive (ts) and has accommodated the chromosomal components of KLF5 stably. In this component the Tn1000 seems to be bonded with metBJF+ argECBH+ operons but their F is lost. Such translocation has occurred in recA strain confirming that Tn1000 together with its chromosomal neighbours (metBJF, argECBH) has become a mobile DNA element.

INTRODUCTION

During 1945-1952, Dr Joshua Lederberg has discovered maleness or fertility factor F in his laboratory strain of *E.coli* (*E.coli* K-12) (1). Subsequently, F factor has been isolated by us for visualization as CCC DNA under electron microscope by modifying the existing procedures and measuring our electron micrographs (2). F is a covalently closed circular (CCC) double stranded DNA and it is nicked for converting the CCC –DNA into an open circular form (2), Our data reports the length of F plasmid as 31um or 94.5 Kb which fully agrees with others (3,4). However, in this article we are still using its length as 94.5Kb instead of 100Kb (5).

Usually, F plasmid integrates into the host E.coli K-12 chromosome, via IS elements IS1, IS2 or IS3 and Hfr strains are stably formed. Occasionally, F excises out again to an extra-chromosomal state with its adjacent chromosomal operon(s) and forms F-prime donors (5,6). Does such integration and excision of F plasmid occur by homologous recombination? In order to answer this question some digression is necessary. Mobile DNA elements, known as IS and Tn DNA elements are present in E.coli K-12 chromosome and most probably such DNA elements have played a role in the evolution of fertility factor F (2, 5). F plasmid has two insertion sequences IS2 and IS3, and one transposon gamma delta or Tn1000. When F integrates into the chromosome via IS2 and IS3, mostly stable Hfr donors are formed but if F integrates via gamma delta the unstable Hfrs (Ra-1,Ra-2 and AB312) are formed (5,6,7). These observations lead us to conclude that integration of F plasmid into its host chromosome via transposon Tn1000 produces unstable Hfr donor but if F reverts back to its extrachromosal state, the stability is restored. Hfr donor becomes F^+ donor without loss of any genetic material. Studies of such unstable Hfr strains (Ra-1, Ra-2, AB313) lead us to conclude that Hfrs formed via transposon Tn1000 IS unstable unlike the stable Hfrs formed by the homologous recombination between their insertion sequences,IS2 and IS3 (RecA –dependent). What is more, the instability of these Hfr donor formed by gamma delta sequence or Tn1000. In this article, we have shown that the F-prime plasmid KLF5 if formed from its unstable Hfr parent Ra-2 by the type II excision method the instability is inherited (2, 8). Until now we have not precisely explained how the integration of F plasmid via its gamma delta sequence takes place without affecting the integrity of Tn1000 (gamma delta). This is very similar to that phage lambda, formation of double lysogen (9). Such duplication of transposon Tn1000 creates instability of

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the host E.coli chromosome! In order to understand such chromosomal instability, we have to walk backward and summarize how we have created antibiotic resistance crisis in the treatment of simple diarrheal disease, Shigellosis in Japan and then in several industrially developed countries (10, 11). In 1971, Rownd *et al* have published an article which demonstrates that R plasmid appeared in an antibiotic resistant pathogen and consists of two components RTF and r-det. These two components have not yet been fully analyzed by taking total information together. We want to address that issue in our next article (in preparation).

Hfrs when formed by the transposon gamma-delta or Tn1000 is highly unstable as reviewed by Low (2, 6). Surprisingly F-primes isolated from such unstable Hfr donors are equally unstable. In support of such observation, Dr. Davidson and co-workers in 1974, also independently observed the F-prime F-14 isolated from the *E. coli* K-12 Hfr strain, AB313 is also very unstable (7). Question remains unanswered what makes them unstable? It is not any coincidence but these two F-primes have been isolated from the Hfr donors in which F has integrated via gamma-delta in the proximity of oriC (E.coli K-12 origin of replication). F-prime KLF5 formed by type II excision method, have gamma-delta sequence probably at both junctions, F plasmid and chromosomal segment carrying metB and argECBH operons. In our 1976 article, we have observed that in such unstable donors, F has integrated into the host chromosome via its transposon Tn 1000 or gamma delta (sfa site of Low). This transposon has been an integral part of *E.coli* K-12 chromosome, located at 88 min and closely linked with metBJF and arg ECBH operons (7, 8). Previous investigators have mostly studied the instability of these F-primes but our objective is to understand the difference between stable and the unstable F-primes. In this work, we have made an attempt to understand how such selfish DNA multiply *in vivo* even without a replicon and translocate in the chromosome without any homology (RecA-independent).

In 1976 article we have already proven that formation of Type-II F-primes via Tn1000 is RecA independent and then the fusion of two F-prime plasmids (one is type I and another one is type II) via gamma-delta (12, 13). In this article, we have shown the translocation of Tn1000 from KLF5 (unstable type II F-prime plasmid) into F-prime trp+ (stable type I F-prime plasmid). Previously, Berg *et al* have shown that inversions and deletions are generated even by a mini Tn1000 (14). However, mini Tn1000 is of length 1Kb approximately and complete Tn1000 is 6Kb but they have not shown whether Hfrs can be formed via mini Tn1000. Major objective is how they multiply even without a replicon. In agreement with previous investigators and our articles already published, we have made it clear that Tn1000 itself and even with additional operon(s) can translocate to get hold of ϕ80att site. This site can be used to form lysogen, for its multiplication and production of transducing phage particle. Finally, gamma delta will multiply as transducing bacteriophage particles (Fig. 4). All these steps are depicted with diagrams and expanded legends.

MATERIALS & METHODS

Materials and methods including bacterial strains have been well defined in our previous publications (2,12).

RESULTS

Type I F-primes, F-prime lac+ and F-prime trp+ are formed by homologous recombination between IS sequences, one on F plasmid and the other one on the chromosome adjacent to trp (15). However formation of such F-primes occur at low frequencies but they are stable. Type –II unstable F-prime is formed by non-homologous recombination (RecA independent) carrying gamma-delta, metB and argE. We have not expanded this information as it is already published in the 1976 MGG article (5). Conclusion was that these two F-prime plasmids were fused resulting in reorganization of chromosomal characters.

We are including some diagrams to demonstrate how Tn1000 or gamma-delta moves from one site to another site in the same chromosome. Precisely, two F-primes are introduced in the same RecA *E. coli* K-12 host Bacteria. F plasmid of KLF5 is eliminated for the incompatibility barrier and leaving behind F-prime trp (KLF155) with ϕ80att.

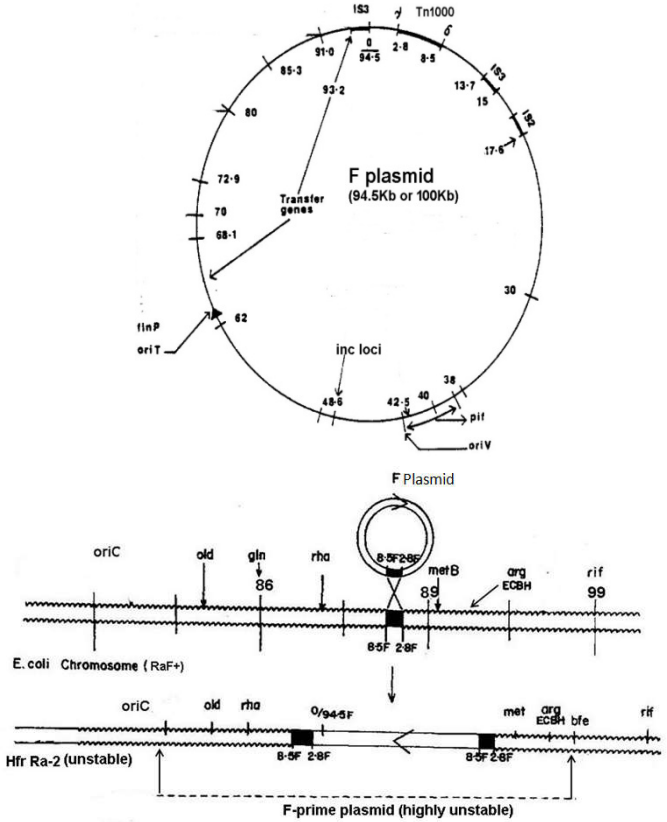

Fig 1 Formation of F-prime KLF 5 with duplication of Tn1000.

Extrachromosomal

Presence of F in the *E. coli* RecA- (RaF+) was observed. Formation of unstable Hfr donor Ra-2 due to integration of F via two gamma-delta or Tn1000 (black solid section) sequences, one on F and another one on the chromosome. Following the formation of F-prime plasmid KLF5. KLF5 is unstable and wants to segregate into two components, F plasmid by itself and a second component carries a chromosomal segment of metB and argECBH flanked by gamma-delta or Tn1000 sequence. F is an autonomously replicating plasmid and stable. KLF5 seggregant carrying metBJF and argECBH operons is lacking replicon but is capable of translocation.

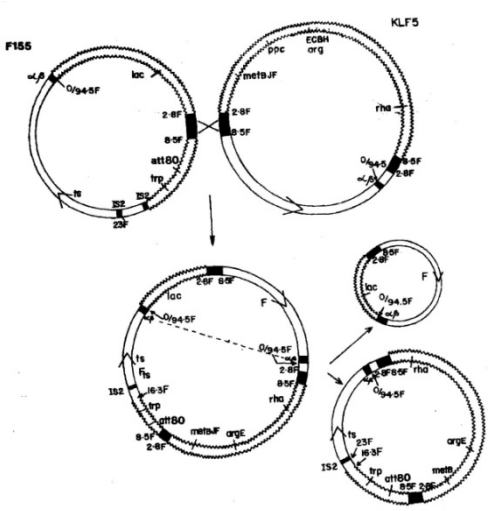

Fig 2. When stable F -prime plasmid KLF155 (F'trp) is introduced into the recipient carrying the unstable KLF5, the F component is eliminated because of incompatibility barrier leaving behind the component carrying "gamma delta metB+argE+ "component, which is not carrying any replicon. This component is rescued by its ability to translocate into the stable plasmid F-prime trp+ with ϕ80 attachment site. Lac carrying component is eliminated for reason of incompatibility. Because of selection pressure, the component carrying "gamma delta metB+argE+ integrates into a site near ϕ80 attachment site as shown diagramatically in Fig.3.

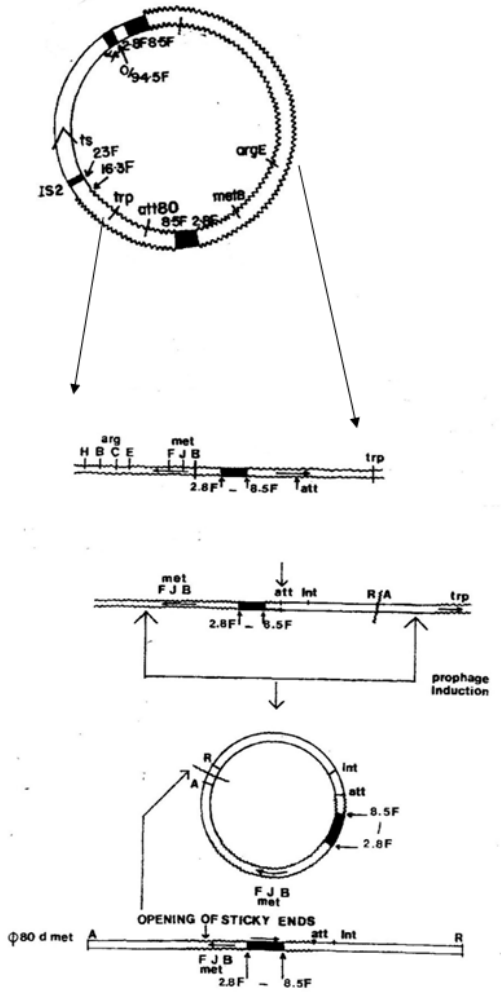

Fig.3. F-prime trp with Gamma-delta transposon transposed at a site proximal to ϕ80 attachment site. We name this F-prime as F-prime trp gamma-delta metBargE. This location is further confirmed by the formation of transducing bacteriophage particle carrying gamma-delta with metB (7). Based on data available now, we schematically show the location of gamma-delta near the phage ϕ80 attachment site in the transducing particle formed. Formation of transducing particle phi 80 d(metB+, gamma-delta) from F-prime gamma-delta trp+metB+ plasmid (16).

DISCUSSION

We should not forget that the location of Gamma-delta is in the proximity of chromosomal origin of replication oriC. It gives us a good reason why E.coli K-12 becomes unstable when Tn1000 is duplicated in Hfr donor Ra-2 by the integration of F plasmid. In order to analyze by EM-heteroduplex technique we have introduced the F-prime plasmid KLF5 formed by type II excision into a RecA host already carrying a stable F-prime plasmid, F-prime trp+.

This is a new interpretation of our article previously assuming a fusion of two participating F-prime plasmids without the knowledge that how these two F-prime plasmids differ. What is in worse, 1972, we had no knowledge that the gamma delta is a transposon capable of moving from one site to another site, not necessarily in the same chromosome. What is more, the Hfrs formed via gamma delta (mobile DNA element) are highly unstable. However, we have not discussed yet why the Hfr donor Ra-2 becomes so unstable (6). This Ra-2 is formed by the duplication of gamma delta and host E.coli K-12 origin of replication (oriC) is not far from the Tn1000 (5.7 Kb length) which is capable of jumping into any site without any sequence homology; but E.coli K-12 does not like such undesirable duplication of gamma-delta near its iC or for a simple reason, its own genetic continuity.

The behaviour of transposon Tn3 and Transposon Tn1000 is similar (17). The gamma-delta carrying component of KLF5 is translocated onto a stable temperature sensitive F-prime trp+ϕ80att by the selection pressure of Trp and Arg, following extensive rearrangement (11). Because of our bias, we have accepted the fusion of two F-prime plasmids to avoid their incompatibility barrier. Instead we found that there was translocation of genetic characters via Tn1000, leaving behind the F. Because KLF5 is unstable it segregates into two components – one is F and the other one is the transposon Tn1000 carrying metB and argE. Under selection pressure of Arg and Trp, these recombinants carrying Tn1000 and metB are selected out. Few stable trans-conjugants (gamma-delta linked to metarg met+arg+trp+) appeared. Transconjugant (met+arg+trp+) outlive following the loss of F-lac+. This loss could be due to their incompatibility behaviour, since Lac operon is also a transposon (18). Because of incompatibility barrier two F-prime plasmids did not co-exist. Instead gamma-delta carrying chromosomal operons metB and argE were transposed onto the site on F-prime trp+. In our previous publications it was thought that F-prime trp+ (KLF155) and KLF5 were fused to form a single replicon. But now we feel that even without replicon fusion transposition of gamma-delta from one plasmid to another may occur. Evidence is lacking if there is any intermediate product consisting of F-prime trp+

and KLF-5 fusion. This work has recorded the translocation of Tn1000 onto a site proximal to the phi80 att site. Ultimate motive of Tn1000 is to multiply and prevail in the genome of transducing bacteriophages at the cost of the bacterial host, thus behaving like a Selfish DNA.

It seems there is a conflict of interest between E.coli chromosomal replicon, (origin of replication or iC located at 84.5 min duplicates bi –directionally) and the transposon Tn1000 which is located at 89 min should not disorganize E.coli K-12 genetic loci. The transposon Tn1000 is a selfish DNA and cannot replicate by itself. Apparently, such a transposon is capable of translocation in the same chromosome without requiring any homology. Tn1000 transposon also satisfies its selfish DNA status. Duplication of Tn1000 (5.7Kb+5.7Kb) is not preferred and hence the Ra-2 Hfr if reverted back to F+ donor the stability is restored. We speculate that the host E.coli K-12 wants Tn1000 to translocate away to the proximity of ϕ80 att site (away from oriC) so that host chromosome stability is not affected. Tn1000 eventually leave the host chromosome by the formation of transducing phage metB or argE.

Subsequently transducing particle is formed which is carrying metB and gamma-delta and the bacteriophage ϕ80 replicon. Such biological activity of gamma-delta or Tn1000 should justify its role as a selfish DNA.

Since 1960 antibiotic resistance transposons have played a major role in shigellosis by defeating our miracle drug ampicillin. But without coming to any solution regarding the crisis, we have used those transposons in *in vitro* gene cloning experiments (19-21). Therefore we should re-assess our knowledge about such undesirable capability of Tn1000 which has ample opportunity to spread if it is still not too late.

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