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Formation of an F′ Plasmid by Recombination between Imperfectly Repeated Chromosomal Rep Sequences: a Closer Look at an Old Friend (F′$_{128}$ *pro lac*)

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Plasmid F′$_{128}$ was formed by an exchange between chromosomal Rep sequences that placed *lac* near *dinB* between many pairs of Rep sequences. Plasmid F′$_{128}$ is critical for selection-enhanced *lac* reversion (adaptive mutation), which requires prior *lac* amplification. The structure of F′$_{128}$ supports the idea that amplification is initiated by Rep-Rep recombination and that general mutagenesis requires coamplification of *dinB* (error-prone polymerase) with *lac*.

Plasmid F′$_{128}$ (*proAB lac*) is a type II F′ plasmid (37) formed by recombination between chromosomal sequences that flank the F plasmid insertion site. F′$_{128}$ was excised from *Escherichia coli* Hfr P804 and was shown genetically to include the entire *lac* operon and the nearby *proA* and *proB* genes but not *proC* (24). The F′$_{128}$ plasmid was widely used to study the *lac* operon (8, 30, 45, 49), mutation and mutagen specificity (9, 29), deletion and inversion formation (1, 38, 42), gene amplification (43, 48), and mechanisms of F-plasmid integration (10, 20).

More recently, F′$_{128}$ has been used in experiments interpreted as indicating that bacteria elevate their general mutation rate in response to selective stress (adaptive mutation) (6, 7, 12, 36, 44). Selection-enhanced reversion requires that the target *lac* operon be located on a conjugative plasmid (18, 33, 34, 40) with an expressed *tra* (transfer) operon (14, 15). General mutagenesis accompanies reversion only when *lac* is on the particular plasmid F′$_{128}$ and is located *cis* to the *dinB* gene (E. S. Slechta, K. Bunny, E. Kofoid, K. Savaraman, S. Gerum, D. I. Andersson, and J. R. Roth, unpublished results). It has been claimed that general mutagenesis is preferentially directed toward the whole F′$_{128}$ plasmid (13). The role of F′$_{128}$ is the least well understood aspect of the adaptive-mutation phenomenology.

The amplification-mutagenesis model (3, 22) proposes that selection has no direct effect on mutation but favors growth of cells with a *lac* amplification. The F′ plasmid contributes to reversion by stimulating *lac* duplication and amplification (40). This alone does not explain why F′$_{128}$ is specifically required for general mutagenesis, why only a subset of *lac* revertants appear to be mutagenized (35), or why general mutagenesis might be more intense on F′$_{128}$ than in the chromosome (13). The structure of F′$_{128}$ reported here suggests answers to these questions.

Original identification of the F′$_{128}$ integration site. Previous work (10) showed that the Hfr strain, from which F′$_{128}$ was formed, arose by recombination between two IS*3* sequences, one in the F plasmid and one in the chromosome. Genetic results demonstrated that the F′$_{128}$ plasmid carries chromosomal genes from both sides of this F integration site and thus was excised from the Hfr chromosome by recombination between chromosomal sequences (24).

Restriction map for F′$_{128}$. A restriction map of F′$_{128}$ was assembled based on available sequence data and pulsed-field gel electrophoresis following digestion with *Bln*I, *Not*I, *Sfi*I, or *Xba*I, assuming that the F plasmid was integrated as described previously (10). This map allowed identification of chromosomal regions within which recombination must have occurred to generate the final plasmid. The sequences of these general regions were examined for repeated elements that might have supported this recombination.

Identification of sequence repeats in regions containing the excision point. Comparison of the two identified regions by using FASTA (32) revealed two pairs of extensive but imperfect repeats. A sequence just clockwise of the *mhp* operon in the *E. coli* chromosome was similar to two different sequences located immediately counterclockwise of the *dinB* gene (Fig. 1). All three tracts turned out to be groups of Rep (repetitive extragenic palindrome) elements (23). Such clusters are also called bacterial interspersed mosaic elements (17). Individual Rep elements are related, imperfectly palindromic 33- to 40-nucleotide sequences that have been placed in three subclasses, Y, Z1, and Z2 (4, 16). These elements frequently appear in clusters that have been designated bacterial interspersed mosaic elements (BIMEs).

The positions of the potential recombination sites are diagrammed in Fig. 1 and were designated based on their approximate position in minutes and the nature and orientation of their subelements. Clusters Rep5.3 and Rep5.4 lie counterclockwise of *dinB* in the *E. coli* genome. Cluster Rep5.3 is of the form (<Y)(Z2>)(<Y) (< and > symbols indicate the orientations of the subelements) and lies immediately counterclockwise of *yafJ*. Cluster Rep5.4 has the structure (Z2>)(<Y)(Z2>)(<Y) and lies immediately counterclockwise of *fhiA*. On the opposite (clockwise) side of *lac*, cluster Rep8.1 has the

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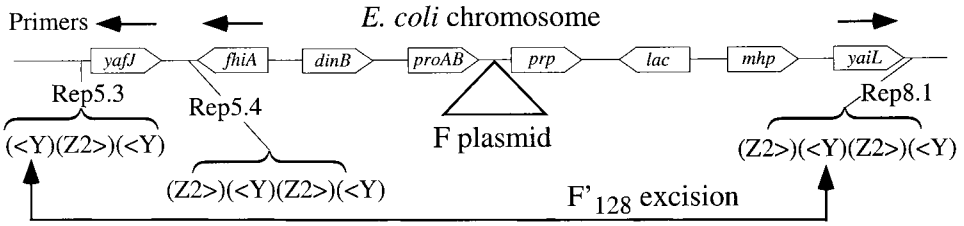

FIG. 1. Sequence repeats flanking *lac* in the *E. coli* chromosome. Triangle, insertion site of the F plasmid, at which two IS3 elements recombined to integrate F and form Hfr P804 (10). The sequences designated Rep5.3, Rep5.4, and Rep8.1 are clusters of Rep sequences whose order and orientation are indicated in parentheses; Y and Z2 are two of the three general types of Rep elements (4). Arrows above the map indicate primers tested for PCR amplification of F'_{128} junction points formed during excision. The pair of direct repeats responsible for F'_{128} excision are indicated below the map. The three Rep clusters were designated R17, R19, and R32 in a previous discussion of these elements (36a).

same structure as Rep5.4 [(Z2>)(<Y)(Z2>)(<Y)] and lies immediately clockwise of the *mhp* and *yaiL* genes. If F′ plasmid excision occurred by simple Rep-Rep recombination, it would require an exchange between Rep8.1 (on the right) and either Rep5.4 or Rep5.3 on the left (Fig. 1).

Identification of the excision point. PCR amplification across both the potential hybrid junctions within F'_{128} was attempted. A very strong signal was found with primers in *yafJ* and *yaiL*. The sequence of the product corresponded to a Rep5.3-Rep8.1 hybrid with a crossover within a 9-bp block of perfect alignment (Fig. 2). The region including the exchange is similar in size to those that support formation of some deletions (11, 47) and duplications (48). The small extent of perfect homology is probably compensated for by the extensive imperfect pairing of nearby sequences.

A weak PCR signal was generated by primers in *fhiA* and *yaiL*, implying that a few cells carry plasmids with the Rep5.4-Rep8.1 junction. It seems likely that the two Rep clusters that remain in the final plasmid after an exchange between Rep5.3 and Rep8.1 can recombine occasionally to generate a deletion that removes about 4 kb and that brings the *fhiA* and *yaiL* sequences close together. The formation of F'_{128} and of this deletion provides evidence that Rep elements can recombine (see below). The whole process of F'_{128} formation is diagrammed in Fig. 3.

The event that formed F'_{128} brought *lac* close to *dinB*. The *dinB* gene carried by F'_{128} encodes an SOS-induced error-prone polymerase (25) that is thought to be responsible for general mutagenesis (adaptive mutation) during *lac* starvation (28, 41, 44). In the chromosome, *lac* and *dinB* genes are separated by over 100 kb. However, on F'_{128} they are separated by only 16.5 kb. On lactose medium, cells with a leaky *lac* mutation can grow if they amplify their *lac* region. The size of the amplified region is generally between 10 and 40 kb (3, 21, 22, 43, 48). The proximity of *lac* and *dinB* on F'_{128} makes it likely that these two genes are at least occasionally coamplified during growth under selection. If *dinB* amplification is a prerequisite for mutagenesis, then a problem regarding DinB-dependent mutagenesis could be resolved.

SOS induction of a single *dinB* gene does not cause mutagenesis in either *Salmonella enterica* (27, 31) or *E. coli* (26). Mutagenesis by DinB has been seen only when the enzyme is overproduced from plasmids (25). This overproduction could be provided by coamplification of *dinB* with the nearby *lac* operon (Slechta et al., unpublished results).

Rep sequences are abundant on F'_{128}. The frequency of Rep elements in the chromosomal region carried by F'_{128} is about fourfold higher than that in the chromosome as whole. (Two of the Rep sequences recombined to form the plasmid.) The frequency of Rep elements near the *lac* operon is particularly high (Fig. 4), and many flanking pairs are oriented so as to support *lac* duplication (arcs in Fig. 4). Roughly 10% of predicted Rep-mediated duplications (inside arcs in Fig. 4) include *dinB* as well as *lac*. This may explain why only about 10% of Lac^+ revertants experience heavy mutagenesis while 90% experience little or none (35). We suggest that the mutagenized *lac* revertants (10%) arise within clones growing with a *lac* amplification that includes *dinB*. Thus most revertants (90%) arise in strains whose *lac* amplification lacks *dinB* and that are not mutagenized; in these clones, reversion is enhanced only by an increase in *lac* copy number. Preliminary results support this possibility (Slechta et al., unpublished results).

Duplications and deletions have previously been shown to form by recombination between Rep elements (2, 39, 46). In the most extensive study, a Rep element between the *hisD* and *hisG* genes of the *S. enterica* histidine operon recombined with a series of distant Rep elements to form a duplication which places the *hisD* gene adjacent to a foreign promoter at each duplication join point (39). It is proposed here that the same event generates the *lac* duplications on F'_{128} that initiate reversion under selection.

Coamplification of *tetA* and *lac* may explain the apparent direction of mutation toward F'_{128}. While selective stress causes very little mutagenesis of chromosomal genes independent of *lac* reversion (5, 44), the claim that it strongly mutagenizes the entire F'_{128} plasmid has been made (13). This was supported by the observation that starvation for lactose enhanced the reversion of a *tetA* frameshift mutation in a Tn*10* element on F'_{128} thought to be located too far from *lac* to be included in any *lac* amplification. During starvation for lactose, a population carrying both the *lac* and *tetA* mutations on F'_{128} was seen to accumulate tetracycline-resistant ($tetA^+$) mutants that had not reverted to lac^+ (13).

We sequenced the Tn*10* insertion used in this experiment and found that it lies within the *mhpC* gene (bp 132), very close to *lac* (Fig. 3). Another Tn*10* insertion in this gene (bp 782) is known to be included in *lac* amplifications that arise under selection (18). Many pairs of Rep elements flank the *mhpC-lac* gene pair (Fig. 4). It seems likely that *tetA* reversion was en-

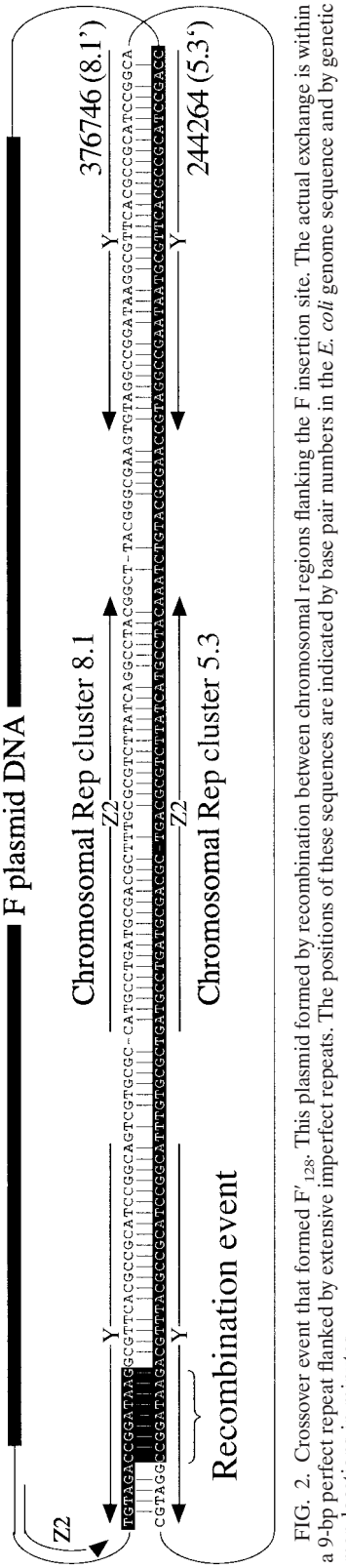

FIG. 2. Crossover event that formed F'_{128}. This plasmid formed by recombination between chromosomal regions flanking the F insertion site. The actual exchange is within a 9-bp perfect repeat flanked by extensive imperfect repeats. The positions of these sequences are indicated by base pair numbers in the *E. coli* genome sequence and by genetic map locations in minutes.

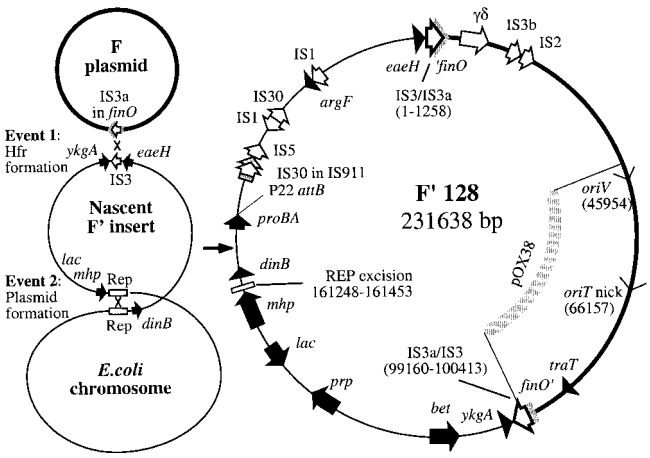

FIG. 3. Formation and final structure of F'_{128}. Filled arrows, genes; open arrows, insertion sequences. Gray arrows designate nested or recombinant insertion sequences. The arc inside the map at the right indicates the extent of pOX38, a minimal F plasmid derivative still capable of both vegetative replication and conjugation (19).

hanced because many clones grew with a *lac* amplification that included the *mhpC*::Tn*10* element (and perhaps *dinB* as well). For such clones, *tetA* reversion frequency would be enhanced by multiple *tetA* copies and possibly by mutagenic overexpression of $dinB^+$. This reversion can occur in clones that have not yet experienced Lac^+ reversion.

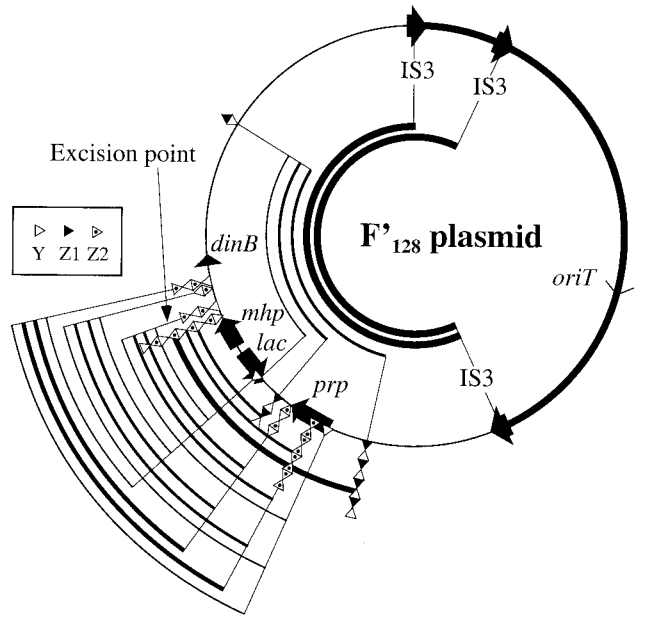

FIG. 4. Distribution of Rep elements on F'_{128} The three classes of Rep elements (Y, Z1, and Z2) are indicated wherever they occur in F'_{128}. While each is an imperfect palindrome, the imperfections are such that a polarity can be assigned to each type. Arrowhead, orientation of each element. All elements with arrowheads pointing away from the center of the circle are in the same orientation; those with arrowheads pointing toward the center are in the opposite orientation. Note that no Rep elements are found within F-plasmid sequences.

Summary. The structure of F'_{128} suggests answers for several questions regarding the adaptive-mutation controversy. Why does selection-enhanced reversion require that the *lac* gene be on a conjugative plasmid (34)? Why must the plasmid genes for conjugative transfer (*tra*) be expressed for optimal *lac* reversion (14, 15)? Why does selection-induced general mutagenesis require that the *lac* mutation be located on the specific plasmid F'_{128} (Slechta et al., unpublished results)? How can DinB cause general mutagenesis in only some of the revertant clones (35)? We propose that the transfer origin of conjugative plasmids generates DNA ends that simulate *lac* amplification (40). The Rep sequences that flank *lac* on F'_{128} may allow frequent amplification. General SOS mutagenesis may rely on the proximity (and coamplification) of the *dinB* and *lac* genes. These special features of F'_{128} may explain why the apparent directed mutation and the induced general mutagenesis seen in the Cairns system are not observed in other genetic systems.

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