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The Stabilization of Episomal Integration by Genetic Inversion: A General Hypothesis

(*E. coli*/recombination/crossover)

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ABSTRACT In F′ cells of *Escherichia coli* K12, recombination in the region of genetic duplication between chromosome and F-genote produces a rapid alteration between the integrated and detached states of the episome. Nevertheless, F′ strains can give rise to Hfr cells in which the F-genote has undergone stable integration. We have found that one such Hfr has retained the genetic duplication as well as the recombination proficiency of the parental F′ strain; to account for the failure of recombination to detach the episome in this Hfr, we have postulated the inversion of one of the duplicated regions. A general model is presented, showing that an inversion must prevent episome detachment by any combination of genetic crossovers.

In secondary F′ cells of *Escherichia coli* K12, in which a segment of DNA is represented both on the chromosome and on the F-genote, crossing over in the region of homology is so frequent that a dynamic equilibrium exists between the integrated and detached states of the episome (Fig. 1). The rate constants for integration and detachment of a small F-genote have been estimated to be 0.1 and 0.9 per cell per generation, respectively (1).

In 1963, Jacob, Brenner, and Cuzin reported that if a secondary F′ is constructed containing a temperature-sensitive F-*lac*, selection for stable Lac$^+$ clones at 42° (the nonpermissive temperature for replication of the autonomous F-*lac*) permits the isolation of rare Hfr strains in which the F-*lac* has become stably integrated into the chromosome (2). The genomic structure that they proposed for such Hfrs is shown in Fig. 2. No explanation was offered for the occurrence of stable F-*lac* integration, but it is clear that the normal process of episomal detachment has been blocked in these strains.

We have used Jacob, Brenner, and Cuzin's technique to isolate a new Hfr strain, PB15, in which a temperature-sensitive F-*gal* (F8-1) is stably integrated into the chromosome (3). The genomic structure of PB15, as a first approximation, is shown in Fig. 3. We have conducted two series of investigations with this strain. One series, reported in our paper (3), showed that during conjugation PB15 undergoes an abnormal process of excision and transfer of chromosomal segments, a process that was revealed thanks to the blockage of normal F-*gal* detachment. The other series, reported below, was designed to discover the nature of that blockage.

Two testable hypotheses readily presented themselves. Since both integration and detachment of an F-genote in a secondary F′ cell are brought about by recombination between duplicated chromosomal regions (e.g., the *gal*$^+$ and *gal*$^-$ regions of PB15), detachment would be blocked by either of two events: mutation to Rec$^-$ (recombination deficient state), or deletion of one of the duplicated regions.

As described below, our experiments unambiguously ruled out both explanations. We were thus left with the puzzle that, although PB15 possesses a duplicated *gal* region and is Rec$^+$ (recombination proficient), the crossing-over that must be assumed to occur between the *gal* regions does not lead to F-*gal* detachment. In our search for a possible explanation, we discovered an interesting topological fact: *if, during or after integration of an episome, one of the duplicated regions undergoes an inversion, the episome becomes permanently trapped.* Crossing-over within such a structure simply reverses the direction of chromosome transfer; detachment cannot occur until a compensating inversion takes place.

In the *Results*, we describe experiments that (*a*) rule out a Rec$^-$ mutation or a deletion as the cause of stable integration in PB15, and (*b*) suggest that it is the original chromosomal *gal*$^-$ region, not the *gal*$^+$ region introduced by the integration of F-*gal*, that is inverted in PB15. The inversion model is presented in the *Discussion*.

MATERIALS AND METHODS

Bacterial Strains. The strains used in this work are all derivatives of *E. coli* K12, and are described in Table 1.

Genetic procedures. The procedures used for the experiments reported below are described in (3).

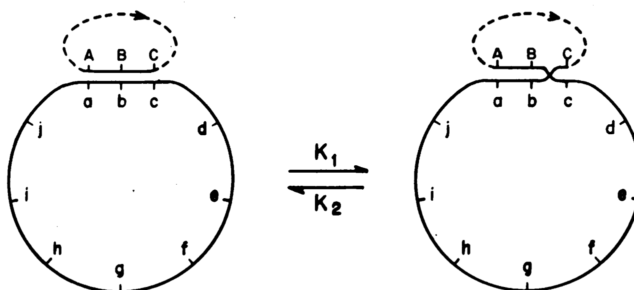

FIG. 1. Dynamic equilibrium between the detached and integrated states of an F-genote in a secondary F′ cell of *E. coli*.

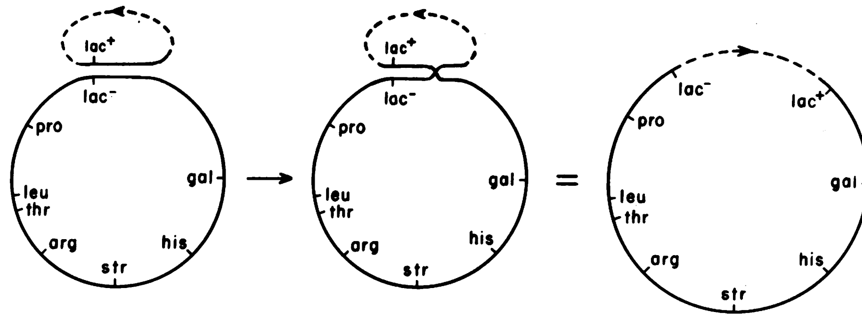

FIG. 2. Structure of the lac^+/lac^- partial diploid Hfr proposed by Jacob *et al.* (2).

RESULTS

The presence of *galT12* in PB15

PB15 is phenotypically Gal^+; if one of its duplicated regions had been deleted, it would necessarily have been that of the original F^- ancestor, carrying the *galT12* mutation. On the contrary, we have been able to confirm the presence of *galT12* in PB15 in two ways:

(a) *By Transfer to gal^+ Recipients.* PB15 was mated with $proC^-$ gal^+ strains AB1515 and χ820, and $proC^+$ recombinants were selected. Of 1300 such recombinants, three were found to be gal^-. This is a very low frequency for inheritance of a proximal marker, and probably results from the close proximity of the transfer origin. Nevertheless, the positive results establish the presence of a gal^- mutation in the early-transferred region of PB15's chromosome.

(b) *By the Appearance of Revertible, Gal^- Segregants.* Both PB15 and its F′ parent, PB71, segregate F^-Gal^- clones at low frequency (3). If PB15 or its parent had undergone deletion of the gal^- chromosomal region, the loss of F-gal^+ should produce female cells with *gal* deletions, rather than point mutations. On the contrary, we have found that PB236, a Gal^- segregant of PB15, is capable of reversion to Gal^+, both spontaneously and after treatment with diethylsulfate or nitrosoguanidine. The reversion frequencies were 1×10^{-8} (spontaneous), 4.6×10^{-6} (diethylsulfate), and 1×10^{-7} (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine).

The recombination-proficiency of PB15

If PB15 were Rec^-, it should be relatively sensitive to killing by ultraviolet light (4). As a more direct test, F^- segregants of PB15 should be able to receive F-genotes but not to integrate markers transferred from an Hfr donor.

Both of these predictions have been tested. PB15 was found to be comparable to Rec^+ strains in its resistance to ultraviolet light (Fig. 4), and an F^- segregant of PB15, when made streptomycin-resistant and used as a recipient in crosses with an Hfr donor, was able to form chromosomal recombinants at a normal rate. Thus, the possibility that PB15 is recombination-deficient has been ruled out.

A direct test of inversion

If we write the order of *gal* genes on the F^- chromosome as $k^+t^-e^+o^+$, then the gene order in PB15, carrying a duplication as a result of F-*gal* integration, must originally have been $k^+t^-E^+O^+$ – (*F DNA*) – $K^+T^+e^+o^+$ (see Fig. 3). Inversion of either of the two regions might then have occurred, producing one or the other of the following structures:

$$(a)\ O^+E^+t^-k^+ - \text{(F DNA)} - K^+T^+e^+o^+$$

$$(b)\ k^+t^-E^+O^+ - \text{(F DNA)} - o^+e^+T^+K^+$$

These two alternative structures for PB15 have been tested (in part) by determination of the orientation of the *gal* operon to the right of the F-DNA. It has been shown that

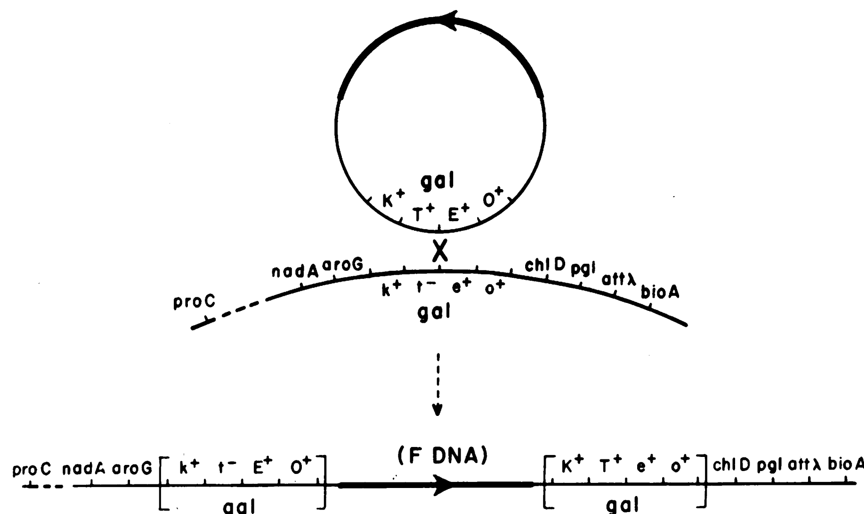

FIG. 3. First step in the formation of Hfr PB15.

induction of λ-lysogens of *E. coli* leads to the "escape synthesis" of the *gal*-determined enzymes (5). In lysogens of *E. coli* that do not carry λdg, escape synthesis presumably occurs by sequential transcription of the *gal* genes in a leftward direction (6). Furthermore, it has been shown that the *gal* operon is normally transcribed leftward (7). Thus, if a λ-lysogen of PB15 is induced, escape synthesis of *gal* enzymes will be observed if PB15 has structure (*a*) above. If, however, PB15 has structure (*b*), no escape synthesis will be observed since the RNA polymerase will now read the non-informational strand of the inverted region.

PB15 was lysogenized with a thermo-inducible strain of λ (λ_cI857), and the synthesis of the epimerase enzyme was measured before and after thermal induction. The rate of escape synthesis was compared with the rate of induction of epimerase by fucose in a nonlysogenic strain of PB15. The results, presented in Table 2, are compared with the results of similar experiments performed with PB501 (an F^- segregant of PB71, the F-prime parent of PB15) and PB236, (an F^- Gal^- revertant of PB15).

Escape synthesis was observed in all three cases. Thus, the orientation of the right-hand *gal* operon in PB15 is $K^+T^+e^+o^+$, and the same orientation is present in the parental and revertant F^- strains.

Thus, if PB15 does carry an inversion, it must be in the left-hand *gal* operon.

DISCUSSION

As an hypothesis to explain the failure of the integrated F-*gal* to detach in PB15, we propose that one of its two *gal*

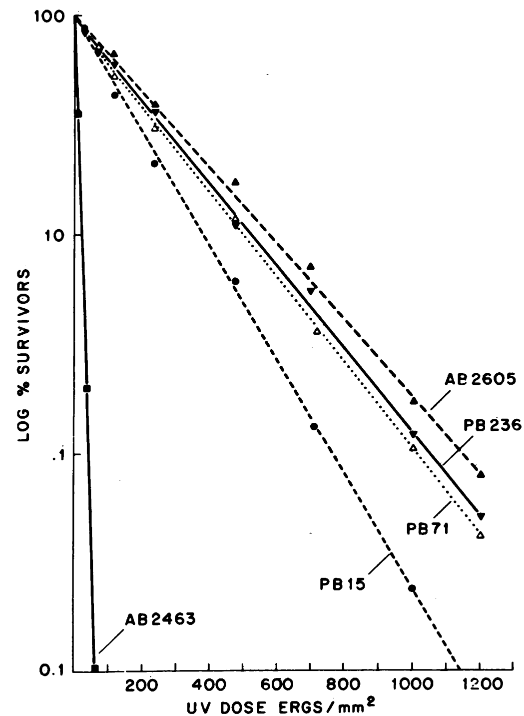

FIG. 4. The effect of ultraviolet-irradiation on PB15 and various control strains. PB71: the F′ parent of PB15; AB2605: the Rec^+, nontemperature-sensitive F′ parent of PB71; PB236: an F^- segregant of PB15; AB2463, a Rec^- strain. See Table 1 for detailed strain descriptions.

TABLE 1. *List of bacterial strains**

Strain no.	Sex	Chromosomal markers*	Other characteristics	Derivation
AB2605	F′	*galT12*	F-gal^+(F8); λ^-	F8 of Hirota and Sneath in W3104 (Lederberg)
PB71	F′	*galT12*	F_{ts}-gal^+(F8-1); λ^-	AB2605
PB15	Hfr	*galT12*	F8-1 integrated; λ^- transfers ← *galT12, purE, tsx.*	PB71
PB236	F^-	*galT12*	Spontaneous segregant	PB15
AB1515	F^-	*thi-1, trpE38, purE42, proC14, leu-6, mtl-1, xyl-5, ara-14, lacY1, tonA23, tsx-67, azi-6, str-109.*		Schwartz (13) strain 13-6.
AB2463	F^-	*thi-1, argE3, his-4, proA2, thr-1, leu-6, recA13, mtl-1, xyl-5, ara-14, galK2, lacY1 tsx-33, str-31.*		Howard-Flanders and Theriot (14)
PB501	F^-	*galT12*	Spontaneous segregant	PB71
PB509	F^-	*galT12*	λcI857	PB236
PB510	F^-	*galT12*	λcI857	PB501
PB511	Hfr	*galT12*	λcI857	PB15
χ820	F^-	*his-53, pyrC30, purE41, thr-16, proC24, xyl-14, cyc-1, tsx-63, str-97.*	λ^-	R. Curtiss

* Strains listed are deposited with the *E. coli* Genetic Stock Center, (CGSC), Department of Microbiology, Yale University, New Haven, Conn. 06510.

† Locus symbols according to Taylor (9). Allele numbers are those assigned by the CGSC.

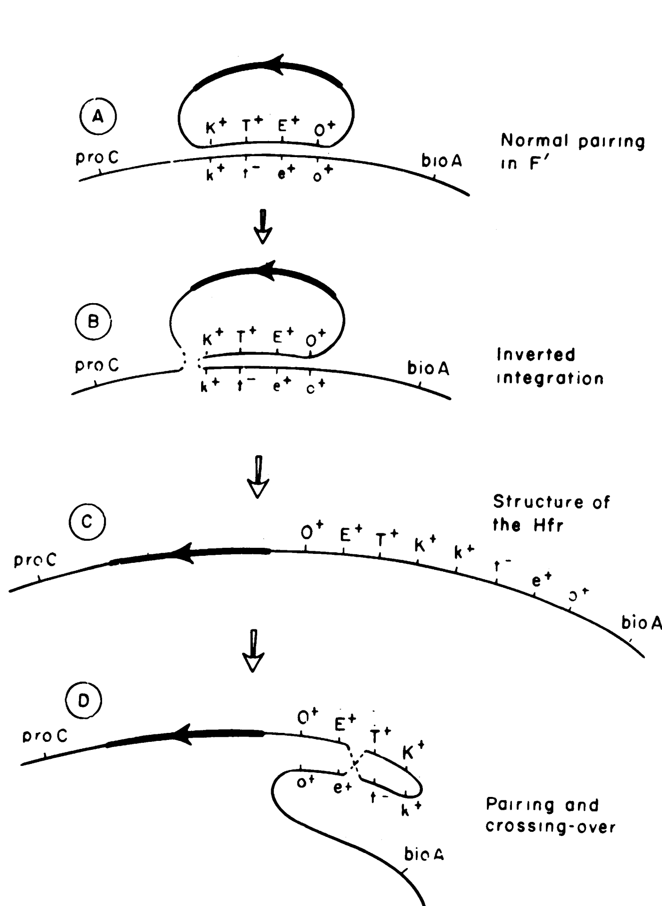

FIG. 5. Formation of a stable Hfr by "inverted integration." See text.

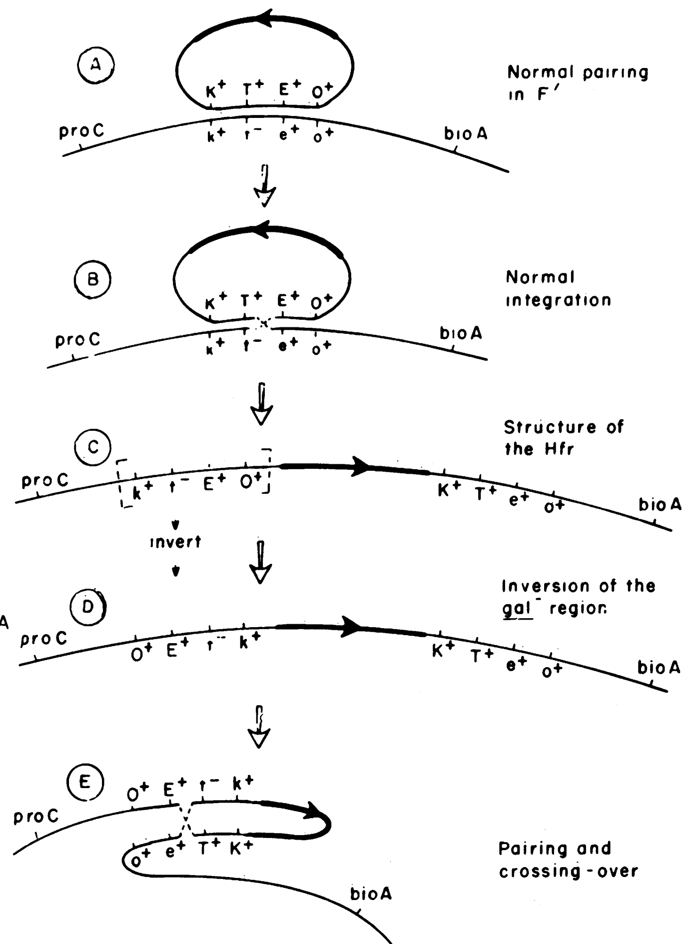

FIG. 6. Formation of a stable Hfr by "postintegrational inversion." See text.

regions has been inverted. Two different types of inversion processes are possible: (*a*) inverted integration and (*b*) postintegration inversion.

(**a**) *Inverted Integration.* We use this term to denote the type of integration shown in Fig. 5. The net effect, for a secondary F′ such as the parent of PB15, is to produce the type of structure shown in Fig. 5*C*. Note that the Hfr produced by such a crossover must transfer its chromosome in the direction opposite to that expected in the parental F′ strain. More importantly, note that any number of crossovers within the region of duplication, whether odd or even, serves only to scramble the order of parental *gal* genes: neither reversal of transfer direction nor detachment will take place. *Detachment can only occur if a second inversion occurs*, giving both regions the same orientation.

(**b**) *Postintegration Inversion.* Integration may occur by a normal crossover, followed by inversion of one of the duplicated regions (Fig. 6). Pairing in such a structure would take the form shown in Fig. 6*E:* note that crossing-over within such a structure, while again failing to produce detachment, may affect the transfer process. An odd number of crossovers will reverse the direction, while an even number will scramble the parental *gal* genes while leaving the transfer direction unchanged. Again, detachment requires the occurrence of a second inversion event.

The structure shown in Fig. 5*C* (inverted integration) can be ruled out for strain PB15, since the great majority of the cells transfer their chromosome in the same direction as the F′ parent (gal^+-------$proA^+$ →). All our observations on PB15 to date, however, are compatible with the inversion structure shown in Fig. 6*D*. Thus, PB15 transfers markers to the right (e.g., gal^+, bio^+, trp^+) as well as to the left of the integrated sex factor, albeit at low frequency. At this time, however, we cannot rule out the formation of the observed recombinant classes by the transfer of enlarged F-genotes, followed by marker integration and sex factor loss, a process that occurs in PB15 (3).

We were able to perform a direct test of inversion of the right-hand *gal* operon in PB15, and to show that it is not inverted. Unfortunately, our method does not permit a direct test of the orientation of the left-hand *gal* operon. Final proof of inversion as the cause of stable F-*gal* integration in PB15 will require a direct demonstration that this operon is inverted.

Genetic inversions do occur in bacteria. For example, the genetic maps produced by Sanderson (8) and Taylor (9) show that the *trp-cysB-pyrF* sequence in Salmonella is inverted with respect to its orientation in *E. coli*, the map orders being otherwise highly congruent. Furthermore, inversion has been shown to occur during F-genote excision (10) and integration (11) in *E. coli*. Finally, Sharp has directly

TABLE 2. *Escape synthesis of UDP-galactose-4-epimerase during lambda phage induction*

A. Nonlysogenic cells grown in minimal medium

Strain	Description	Epimerase activity* Without fucose	Epimerase activity* With fucose†	Ratio
PB15	Hfr	1.84	8.33	4.5
PB236	F^- segregant of PB15	0.61	6.93	11.4
PB501	F^- segregant of PB71 (the F' parent of PB15)	0.41	8.82	21.5

B. Lysogenic cells grown and thermally induced‡ in minimal medium

Strain	Description	Epimerase activity* λ not induced	Epimerase activity* λ induced	Ratio
PB511	PB15 (λcI857)	0.85	1.40	1.65
PB509	PB236 (λcI857))	0.35	0.74	2.1
PB510	PB501 (λcI857)	0.24	1.58	6.6

C. Lysogenic cells grown and thermally induced‡ in broth

Strain	Epimerase activity* λ not induced	Epimerase activity* λ induced	Ratio
PB511	0.675	1.87	2.8
PB509	0.165	0.92	5.6
PB510	0.12	1.11	9.25

* Units of activity per mg of protein. One unit is the amount producing 1.0 μmol of UDP-glucose per hr. Assay performed according to the method of Wilson and Hogness (15).

† Fucose (an inducer of the *gal* operon) was added at a final concentration of 5 mM.

‡ 10 min at 42°, followed by 30 min at 37°.

demonstrated the presence of inverted duplications in *E. coli* DNA, using the techniques of molecular hybridization and electron microscopy (12). Thus, the trapping of integrated episomes by appropriate inversions may be expected to have occurred, and to have played a role in the evolution of bacterial genomes.

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