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Abnormal Excision and Transfer of Chromosomal Segments by a Strain of *Escherichia coli* K-12

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PB15 is an Hfr strain of *Escherichia coli* K-12. It arose from an F′ strain carrying a temperature-sensitive F-*gal* by an event which blocked the detachment of F-*gal* in the normally reversible integration process. In PB15, the detachment of F-*gal* by a second mechanism can now be detected: this mechanism results in the excision and transfer of extended chromosomal segments which include the integrated F-*gal*; the excised segments are inferred to have circularized. Their excision, which is independent of the *recA*$^+$ allele, occurs at an unusually high rate during conjugation; a mutant F-initiator protein is suggested as the cause of this phenomenon. After their establishment in recipients, the enlarged F-genotes undergo further deletions of included donor genes by a process which is again *recA*$^+$-independent. In Rec$^+$, but not in Rec$^-$, cells, a high proportion of the deleted fragments are rescued by integration into the recipient's chromosome.

The discovery by Jacob et al. (12) of strains of *Escherichia coli* K-12 in which a normal and a temperature-sensitive replicon have become fused to form a single genetic element raised some interesting questions concerning the control of chromosomal replication and transfer. To initiate our own studies of this process, we used the procedure of Jacob et al. to select a strain in which a temperature-sensitive F-*gal* (F8-1) had become stably integrated into the bacterial chromosome. F8-1 was introduced into an F$^-$ strain carrying the point mutation *galT12*, and selection was made for stable Gal$^+$ clones at 42 C. A number of such clones were isolated and tested for Hfr behavior; of these, one was selected which transfers the *pro-thr-leu* region of the chromosome early and the integrated episomal *gal*$^+$ marker late. The inferred structure of this Hfr, called PB15, is shown in Fig. 1.

As we began our studies on chromosome transfer by PB15, we became aware of an unexpected behavior of the *gal*$^+$ locus. According to the structure shown in Fig. 1, this locus should not be transferred as part of the chromosome until approximately 90 min, at a frequency of less than 0.1%. Furthermore, the frequency of F′ revertants in the culture, possessing an autonomous temperature-sensitive F-*gal*, was less than 0.1%, so that autonomous F-*gal* transfer should not have exceeded this figure. Contrary to these predictions, however, PB15 was found to transfer *gal*$^+$ at frequencies of 2% or higher. An analysis of this phenomenon has revealed that the *gal*$^+$ locus is transferred as part of a genetic element which includes not only the original temperature-sensitive F, but also variable lengths of adjacent chromosomal deoxyribonucleic acid (DNA), extending as far as the *xyl* locus in one direction and the *his* locus in the other.

Thus, PB15, in which normal detachment of the integrated F-*gal*$^+$ is blocked, is able to excise segments of the chromosome which—if they include the original F-*gal* episome—may be transferred as independent genetic elements. In the present paper, we describe experiments which (i) demonstrate this phenomenon, (ii) characterize the excised elements, and (iii) bear on the nature of the abnormal excision process.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work are all derivatives of *E. coli* K-12 and are described in Table 1. AB2605 is an F′ strain in which

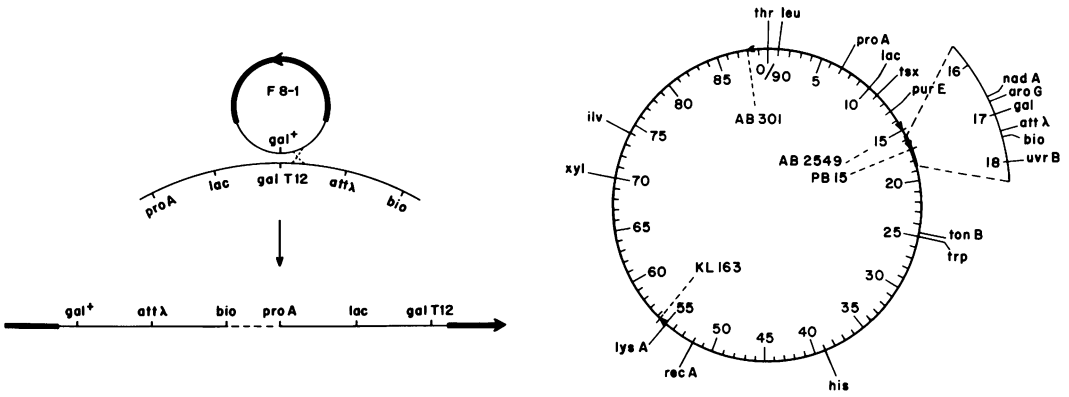

FIG. 1. *(left) Derivation of Hfr strain PB15. (right) Locations of loci, and points of origin of Hfrs.*

TABLE 1. *List of bacterial strains*[a]

Strain	Mating type	Chromosomal markers[b]	Other properties	Derivation
AB2605	F′	*galT12*	F-*gal*$^+$ (F8); λ$^-$	F8 of Hirota and Sneath in W3104 (Echols)
PB71	F′	*galT12*	F_{ts}-*gal*$^+$ (F8-1); λ$^-$	AB2605
PB15	Hfr	*galT12*	F8-1 integrated; λ$^-$; transfers ← *galT12*, *purE*, *tsx* - - -	PB71
PB146	Hfr	*galT12*	F8-1 integrated; λ$^+$	PB15
PB354	Hfr	*galT12*, *recA1*, *nalA12*	F8-1 integrated; λ$^-$	KL163 × PB15
PB501	F$^-$	*galT12*		PB71
AB301	Hfr	*metB1*, *rel-1*	Transfers ← *thr*, *leu*, *proA* - - -	P4× (F$^+$) (Jacob)
AB2549	Hfr	*metE46*, *tonA22*, *rel-1*	Transfers ← *purE*, *tsx*, *lac* - - -	K-10 (from A. Garen)
AB3511	F$^-$	*thi-1*, *ilvD188*, *his-4*, *proA2*, *trp-3*, *mtl-1*, *malA1*, *ara-9*, *galK2*, *lacY1* or *lacZ4*, *ton-1*, *tsx-3*, *sup-38*(amber), *str-8* or *str-9*	λ$^-$(?), λ^R	AB1976 (Eggertsson)
KL163	Hfr	*recA1*, *thi-1*(?), *drm-3*, *nalA12*	Transfers ← *lysA*, *thy*, *recA* - - -	KL16-99 (K. B. Low)
W2961	F$^-$	*thi-1*, *thr-1*, *leu-6*, *proA2*, *lacY1*, *galK2*, *mtl-1*, *xyl-5*, *ara-14*, *strA20*	λ$^-$	W2915 (Lederberg)
AB712	F$^-$	Same as W2961	λ$^+$	W2961
PB69	F$^-$	Same as W2961	λ$^-$, λ^R	W2961
PB68	F$^-$	Same as W2961	λ$^+$, λ^R	W2961
PB349	F$^-$	Same as W2961, plus *recA1*, *nalA12*	λ$^+$, λ^R	KL163 × PB68
PB440	F$^-$	*proC14*, *ilv-290*, *str-128*, deletion of segment *nadA-uvrB*		Streptomycin-resistant derivative of CC70793 (N. Schwartz); deletion isolated by method of Adhya et al. (4)

[a] Strains listed are deposited with the *Escherichia coli* Genetic Stock Center, Department of Microbiology, Yale University, New Haven, Conn.

[b] Locus symbols according to Taylor (16). Allele numbers are those assigned by the Genetic Stock Center.

the F-genote (F8) contains the sex factor and the genes for galactose metabolism. The temperature-sensitive F-genote (F8-1) in PB71 was derived from F8 by mutation as described below; the Hfr strain PB15 is isogenic with PB71, except that F8-1 has been integrated at a point in the chromosome between *lac* and *bio*. The order of markers transferred by PB15 is, for the majority of donor cells, origin-*galT12-lac-proA-thr-leu-xyl-bio-gal*$^+$.

Media. The media routinely employed have been described previously (1). The complete medium used was L broth (15).

Mating conditions. Uninterrupted crosses in liquid media were performed by the method of Adelberg and Burns (1) with a ratio of donor to recipient cells of 1:20. Mating on plates was carried out by techniques similar to the cross-streak test of Berg and Curtiss (6). Interrupted matings were performed by the technique described by Taylor and Thoman (17), with the use of the vibratory device of Low and Wood (14) to terminate chromosome transfer prior to plating.

Scoring of unselected markers. Recombinants were examined for their inheritance of unselected markers by patching at least 200 colonies to the appropriate selective agar, incubating overnight, and replica-plating to various agars specific for the unselected markers. The sex of recombinants was scored by replicating the patch-plates to thick EMB-O agar plates (10) spread with 0.1 ml of 20% glucose and about 10^9 particles of the male-specific phage R17. Female cells grow up as pale pink patches on EMB-O plates, whereas male patches are dark red with a pale pink edge. In some experiments, recombinants were grown up overnight in L broth and were tested for their ability to give plaques when infected with either R17 or the female-specific phage ΦII.

Acridine orange curing of F-genotes. Acridine orange curing was performed essentially by the method of Hirota (11) except that the cells were grown with acridine orange in L broth adjusted to *p*H 7.6. Cultures to be treated with acridine orange were derived from single colonies.

Isolation of the temperature-sensitive F-gal$^+$. The F′ strain AB2605 was treated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine according to Adelberg et al. (2). Survivors were grown in broth at 30 C to allow segregation; suitable dilutions were then spread onto MacConkey-galactose agar and incubated overnight at 30 C. Plates were replicated to MacConkey agar at 30 and 42 C, and, after the colonies had grown up, were examined for temperature sensitivity of Gal$^+$ and maleness at 42 C. One such colony that cured well at 42 C was selected, and the F-genote was transferred back into a strain with the same chromosomal background as AB2605 (and which had not been exposed to mutagen) by way of a secondary recipient with suitable contraselective markers. The final strain is designated PB71; the F-*gal* which it carries, and which was confirmed to be temperature-sensitive, is designated F8-1.

Isolation of strain PB15. The parental F′ strain PB71 carries the temperature-sensitive F-genote F8-1; it is Gal$^+$ and male at 34 C but Gal$^-$ and female after growth at 42 C. Colonies that were Gal$^+$ at 42 C were isolated from PB71. Several hundred such isolates were screened for their ability to transfer chromosomal markers to the recipient strain AB712 at 34 C. PB15 was one of a number of isolates that transferred *proA*$^+$ at high frequency and *gal*$^+$ at low frequency, as shown by cross-streaking tests on plates.

RESULTS

Transfer of gal$^+$ by PB15. When PB15 was isolated, we were interested in the effect of the temperature sensitivity of F-replication on the transfer of genetic markers during conjugation. Consequently, the formation of recombinants between PB15 and several recipient strains was examined at 34, 37, and 42 C.

Preliminary experiments at the three temperatures showed that PB15 exhibited a relatively high level of transfer of the *gal*$^+$ allele. Table 2 compares the numbers of recombinants when PB15 was grown and mated with F$^-$ strain W2961 at 34 C. Also included in Table 2 are the results from similar crosses carried out under identical conditions with two well-characterized Hfr strains. It is apparent from the data in Table 2 that PB15 forms recombinants for *gal*$^+$ with W2961 with a frequency similar to that found for a Hayes-type Hfr (AB301), although it gives rise to recombinants for *pro*$^+$ and *thr*$^+$-*leu*$^+$ at frequencies similar to those of a Cavalli-type Hfr (AB2549).

The results in Table 2 suggested that the F-factor from *F-gal*$^+$ had been integrated into the chromosome of PB15 to give a point of origin and direction of chromosomal transfer similar to a Cavalli-type Hfr, but that the *gal*$^+$ marker was no longer linked to the chromosomal (integrated) F-factor. Time-of-entry experiments established that the point of origin of PB15 was similar to that of AB2549 (Fig. 2). Experiments conducted at 34 and 37

TABLE 2. *Recombinant formation by Hfr donors at 34 C*[a]

Donor strain	Recipient	*pro*$^+$	*thr*$^+$-*leu*$^+$	*gal*$^+$	*xyl*$^+$
PB15	W2961	23.4	13.1	3.7	0.5
AB301[b]	W2961	11.8	20.0	2.4	0.01
AB2549[c]	W2961	27.5	15.8	0.005	1.2

[a] Recombinant frequencies are given as percentages of the number of male cells in the mating mixtures. At the commencement of mating, the number of male cells was 10^7 to 2×10^7/ml, and of female cells, 10^8 to 2×10^8/ml.

[b] Independently isolated Hfr in which chromosome transfer is similar to that of the Hayes Hfr.

[c] Derivative of Hfr Cavalli.

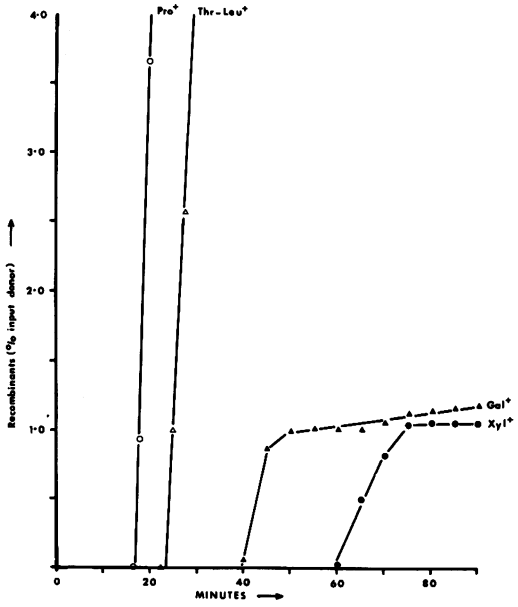

FIG. 2. *Kinetics of recombinant formation in a cross of PB15 × PB68 at 37 C.*

C showed that the relative distances between *pro*$^+$, *thr*$^+$-*leu*$^+$, and *xyl*$^+$ were essentially the same for PB15 and AB2549. No *gal*$^+$ recombinants were recovered in any mating with AB2549 (the Cavalli-type Hfr) as donor, but the *gal*$^+$ marker from PB15 entered the recipient before *xyl*$^+$ at 34 C and after *xyl*$^+$ at 42 C. We have found the actual time of entry of the galactose marker of PB15 to vary in a non-reproducible manner from experiment to experiment, despite the reproducible times of entry of *pro*$^+$, *thr*$^+$-*leu*$^+$, and *xyl*$^+$.

The time-of-entry results could be interpreted to mean that *gal*$^+$ has been transposed to some other location on the chromosome, or that PB15 is a double male (7). However, an analysis of the linkage relationships of the unselected markers in the several classes of recombinants obtained from a standard 60-min broth cross suggested that neither explanation accounted for the behavior of the *gal*$^+$ marker. The data in Table 3 show that *gal*$^+$ is more closely linked to *pro*$^+$ and *thr*$^+$-*leu*$^+$ than it is to *xyl*$^+$, and that the majority of the *gal*$^+$ recombinants are male. This latter result was confirmed by examining the genetic constitution of the several recombinant classes as a function of time in an interrupted mating experiment. The data presented in Table 4 show that the majority of the *gal*$^+$ recombinants are male from the earliest times. These results indicate that PB15 is not a double male, nor does it possess a transposition of *gal*$^+$ to a site on the chromosome between *xyl* and *thr-leu*.

Characterization of the transferred gal$^+$ element. The genetic constitution of the major classes of *gal*$^+$ recombinants from PB15 by PB68 matings are shown in Table 5. Five to 12 colonies from each class were spread on MacConkey-galactose plates, incubated at 34 and

TABLE 3. *Unselected marker analysis of recombinants from a 90-min cross of PB15 × PB68 at 34 C*

Selected marker	Percentage of recombinants which inherited				
	pro$^+$	*thr*$^+$-*leu*$^+$	*xyl*$^+$	*gal*$^+$	♂
pro$^+$	(100)	47	0	2	2
thr$^+$-*leu*$^+$ ..	77	(100)	1	0	0
xyl$^+$	38	27	(100)	0	0
gal$^+$	20	13	1	(100)	82

TABLE 4. *Unselected marker analysis of recombinants in an interrupted mating experiment between PB15 and PB68 at 37 C*

Selected marker	Time (min)	Percentage of recombinants which inherited				
		pro$^+$	*thr*$^+$-*leu*$^+$	*xyl*$^+$	*gal*$^+$	♂
pro$^+$	20		1	0	0	0
	30		39	0	0	0
	40		35	0	0	0
thr$^+$-*leu*$^+$.	25	71		0	2	0
	30	69		1	1	0
	40	71		0	2	0
xyl$^+$	45	42	23		2	2
	50	37	19		0	0
	60	43	31		2	0
	70	39	28		0	0
	80	38	25		2	0
gal$^+$	65	9	0	0		78
	70	19	19	2		91
	80	29	21	0		91
	90	13	9	1		93

TABLE 5. *Genetic constitution of the major classes of gal$^+$ recombinants from a mating of PB15 and PB68 at 34 C*

Class	Percentage of total *gal*$^+$ recombinants
gal$^+$ *proA2 thr-4 leu-8 xyl-5*♂	32.0
gal$^+$ *proA*$^+$ *thr*$^+$-*leu*$^+$ *xyl-5*♂	25.0
gal$^+$ *proA*$^+$ *thr-4 leu-8 xyl-5*♂	13.0
gal$^+$ *proA2 thr*$^+$-*leu*$^+$ *xyl-5*♂	9.0
gal$^+$ *proA*$^+$ *thr*$^+$-*leu*$^+$ *xyl*$^+$♂	2.5
All other classes	18.5

42 C, and replicated to EMB-O plates spread with phage R17 to test for maleness. The majority of the recombinant colonies were Gal$^+$ and male at 34 C and Gal$^-$ and female at 42 C. Occasional recombinants were Gal$^+$ and male at both temperatures. The latter were not curable by acridine orange at either 34 or 42 C. Since the Gal$^+$ recombinants that were temperature-sensitive at 42 C were acridine orange-curable at 34 C, we presume that the stable Gal$^+$ recombinants are strains in which the F-genote had become reintegrated.

We also examined the temperature sensitivity of the *gal*$^+$ marker in F$^-$ recombinant classes equivalent to the male classes in Table 5. None of the female *gal*$^+$ recombinants from the cross of PB15 × PB68 at 34 C was Gal$^-$ at 42 C.

The evidence presented above suggested that in over 2% of the matings in a cross of PB15 × PB68, the donor transfers a temperature-sensitive F-*gal*$^+$ to the recipient. We compared the behavior of PB15 as a donor of *gal*$^+$ with two F-prime strains with which it is isogenic: AB2605, which carries wild-type F-*gal*$^+$ (F8), and PB71, which contains the temperature-sensitive F-*gal*$^+$ (F8-1). Gal$^+$ hybrids from matings between AB2605 and PB68 were Gal$^+$ and male at 34 and 42 C, whereas the Gal$^+$ hybrids isolated when PB71 was the donor were Gal$^+$ and male at 34 C but Gal$^-$ and female at 42 C. The major difference between PB15 and the strains carrying the F-genotes was the level at which chromosomal markers were transferred to the recipient strain. Up to 50% of the Gal$^+$ hybrids formed by PB15, but less than 0.1% of the Gal$^+$ hybrids formed by PB71 or AB2605, were also Pro$^+$.

We conclude from the experiments described above that PB15 transfers both chromosomal markers and a temperature-sensitive F-*gal*$^+$ to PB68. We then asked the question: what is the relationship of transferred chromosomal markers, such at *pro*$^+$, to the transferred F_{ts}-*gal*$^+$? Is the chromosomal segment attached to the F-genote or is it autonomous?

Instability of the donor genetic material in the recipient. From the data presented above, it would be reasonable to expect that the *pro*$^+$ and *thr*$^+$-*leu*$^+$ markers would follow the fate of the F_{ts}-*gal*$^+$ merogenote if they were part of the same replicon. We tested the recombinant classes shown in Table 5 by spreading them to MacConkey galactose plates and to EMB-O plates spread with R17. The plates were incubated at 34 and 42 C.

Four of five isolates from the *gal*$^+$-*pro*$^+$-*thr*$^+$-*leu*$^+$-*xyl*$^+$ male recombinant class segregated large numbers of Gal$^-$ and sectored colonies at 34 C. All Gal$^-$ segregants were female, and contained various combinations of the *pro*$^+$, *thr*$^+$-*leu*$^+$, and *xyl*$^+$ markers.

All Gal$^+$ isolates were male, and they, too, could have any combination of donor chromosomal markers. Individual Gal$^+$ isolates continued to segregate Gal$^-$ colonies on repeated culture, and they continued to segregate Pro$^-$, Thr-Leu$^-$, and particularly Xyl$^-$ colonies, as long as they were Gal$^+$ and male. All isolates were Gal$^-$ at 42 C, and, although they could have any combination of the *pro*$^+$, *thr*$^+$-*leu*$^+$, and *xyl*$^+$ markers, they no longer segregated the chromosomal markers on repeated subculture.

Similar results were found with the other Gal$^+$ male recombinant classes shown in Table 5. The Gal$^+$ recombinant classes that were female were tested in a similar fashion; instability of either chromosomal markers or Gal$^+$ was never found with these recombinants.

The results described above suggested that the loss of F-*gal*$^+$ was dependent on temperature, but that the instability of the *pro*$^+$ and *thr*$^+$-*leu*$^+$ markers was independent of temperature. This supposition was tested by growing a male *gal*$^+$-*pro*$^+$-*thr*$^+$-*leu*$^+$-*xyl*$^-$ recombinant that was known to be unstable for *pro*$^+$-*thr*$^+$-*leu*$^+$ at 30 C. After overnight growth, the culture was diluted 1:100 into four flasks of L broth. The flasks were incubated overnight at 30, 34, 37, and 42 C. The contents of each flask were diluted and spread to L plates which were incubated overnight at the same temperature as the liquid culture. The colonies that grew up on the L plates were replicated to agars selective for Pro$^+$, Thr$^+$-Leu$^+$, Gal$^+$, and maleness, and were incubated for 24 to 48 hr at the appropriate temperature corresponding to the growth temperature of the original liquid culture.

Figure 3 shows that although some cells have lost the *pro*$^+$-*thr*$^+$-*leu*$^+$ markers at 30 C, the loss of these chromosomal markers is independent of temperature, whereas the loss of Gal$^+$ and maleness is a function of increasing temperature. In other experiments with this isolate, we showed that the stability of the *pro*$^+$-*thr*$^+$-*leu*$^+$ markers was unaffected by exposure to acridine orange under conditions that converted 90 to 95% of the cells to the Gal$^-$ female condition.

We have examined the stability of markers transferred by PB15 to PB68 in several experiments in which *pro*$^+$, *thr*$^+$-*leu*$^+$, or *xyl*$^+$ was the selected marker. Irrespective of the genetic constitution of the recombinants, we did not

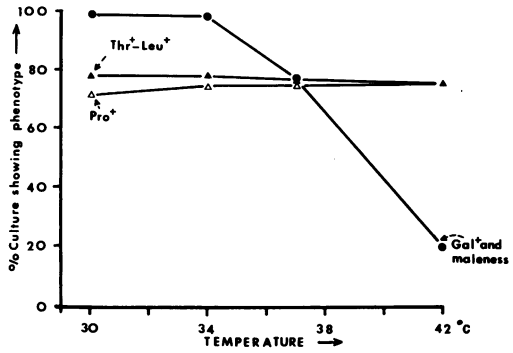

FIG. 3. *Effect of temperature on the instability of markers in a male recombinant. See text for explanation.*

observe segregation for any chromosomal markers provided they had not inherited *gal*$^+$ and maleness. The infrequent recombinants which had inherited *gal*$^+$ and maleness could be cured of these two markers by exposure to 42 C, and they showed segregation of the other donor markers in the manner shown by the recombinants selected for the inheritance of *gal*$^+$.

Linkage of F_{ts}-gal$^+$ to the adjacent pro-thr-leu segment. At first glance, the results presented above suggest that the *pro*$^+$-*thr*$^+$-*leu*$^+$-*xyl*$^+$ segments are not covalently linked to F_{ts}-*gal*$^+$. If so, it should be possible to isolate classes that have lost F_{ts}-*gal*$^+$ but have retained the unstable *pro*$^+$-*thr*$^+$-*leu*$^+$ segment; conversely, classes that have lost *pro*$^+$-*thr*$^+$-*leu*$^+$ but have retained the temperature-sensitive F-*gal*$^+$ should also be found. We looked for the former class of segregant, but found that, if it occurs at all, it must be present at the level of less than 1 in 10^4 colonies. Many isolates of the latter class of segregant, on the other hand, have been found.

We examined Gal$^-$-Pro$^+$-Thr$^+$-Leu$^+$ female colonies from the experiment shown in Fig. 3 to see if reintroduction of the temperature-sensitive F-genote increases the frequency of segregation of the *pro*$^+$-*thr*$^+$-*leu*$^+$ markers. Five isolates that had lost F_{ts}-*gal*$^+$ were mated with PB71, which carries the F_{ts}-*gal*$^+$ episome and is the parental strain of PB15. None of the colonies from any of the reinfected isolates showed segregation of the *pro*$^+$-*thr*$^+$-*leu*$^+$ markers, although, as expected, the *gal*$^+$ marker was temperature-sensitive and susceptible to curing by acridine orange.

The experiments described thus far are compatible with two alternative hypotheses concerning the state of the transferred chromosomal segments (e.g., *pro*$^+$-*thr*$^+$-*leu*$^+$): (i) F_{ts}-*gal*$^+$ and the chromosomal segment are transferred as two separate elements, but the latter is maintained in the recipient in the autonomous state only if F_{ts}-*gal*$^+$ is also present; or (ii) a single element is transferred, consisting of F_{ts}-*gal*$^+$ linked to an adjacent segment of chromosome; once in the recipient, the chromosomal element undergoes a high rate of detachment, either becoming integrated into the recipient's chromosome or else lost. We favor the latter hypothesis as the simplest one which fits the observed data.

Linkage of gal$^+$ to the λ attachment site. In some preliminary experiments designed to locate the point of origin of PB15, we found that a λ lysogen of PB15 (PB146) mated with PB69 (isogenic with PB68 but λ$^-$) gave zygotic induction at a much earlier time than expected for chromosomal transfer of the λ attachment locus. Conjugation experiments in which PB146 was mated with PB68 to measure the time of transfer of *gal*$^+$ and with PB69 to measure the time of zygotic induction suggested that λ was transferred at the same time as *gal*$^+$. In an interrupted mating experiment conducted at 34 C with PB69 as recipient, zygotic induction was observed to begin at 40 min after mating, and about 2 to 4% of the donors transferred λ to the recipient. In parallel experiments, the *gal*$^+$ marker was shown to be transferred to PB68 at about the same time and to half the level as λ (Fig. 4).

These experiments suggested that markers in a clockwise direction from the presumed site of integration of the F_{ts}-*gal*$^+$ could be transferred to a recipient as part of the excised episome. To test this suggestion, *gal*$^+$ recombinants were selected from a mating of PB15 with the F$^-$ strain PB440, which carries a dele-

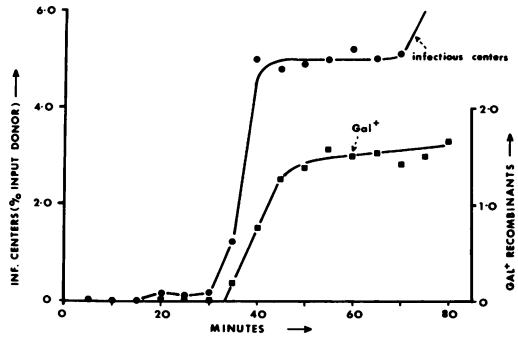

FIG. 4. *Kinetics of zygotic induction and recombinant formation in a cross of PB146 (λ-lysogenic derivative of PB15) with PB68 (for gal transfer) and with PB69 (for zygotic induction).*

tion of the *nadA-uvrB* region (including the λ attachment site). All of the *gal*$^+$ recombinants were male, and all possible combinations of *pro*$^+$, *nadA*$^+$, *aroG*$^+$, *bio*$^+$, and *uvrB*$^+$ were present as unselected markers, although 0 of 200 were *ilv*$^+$. Representative samples of each class of recombinant were examined for temperature sensitivity and for the segregation of unselected markers. All were temperature-sensitive for *gal*$^+$ and maleness, and, as before, the cultures tended to segregate *pro*$^-$*aroG*$^-$-*nadA*$^-$*uvrB*$^-$ and *bio*$^-$ colonies at both 34 and 42 C. There were occasional *gal*$^+$ sectors in some colonies incubated at 42 C, suggesting that reintegration of F'$_{ts}$-*gal*$^+$ had occurred.

A number of recombinants were also tested for their ability to transfer λ prophage. Samples from cultures of representative recombinants were exposed to an excess of λ particles, and survivors were isolated as putative lysogens. The isolates were tested for the presence of λ prophage by the method developed by Gottesman and Yarmolinsky (10) for detecting λ*int* mutants. Isolates which were *gal*$^+$*bio*$^+$*uvrB*$^+$ did not show lysis when stabbed to EMB-O plates spread with λb2 and were scored as lysogenic, whereas isolates that were *gal*$^+$*bio*$^-$*uvrB*$^-$ did show lysis and were scored as nonlysogenic. The F-genotes from both classes of isolates were transferred to PB501 with *ilv*$^-$ as a contraselective marker, and the resulting F-ductants were tested for their ability to produce zygotic induction in matings with PB69. In each case in which the isolate appeared to be λ-lysogenic by the stab test, zygotic induction was observed, whereas the nonlysogens never gave zygotic induction.

From these experiments, it appears that the λ attachment site as well as the *bio* and *uvrB* genes, all of which are clockwise to the presumed site of integration of F8-1, can be included along with *gal*$^+$ in the episome excised on mating PB15 with a recipient. We examined the extent of excision of markers in a clockwise direction by mating PB15 with AB3511. We found 0.01% *gal*$^+$*trp*$^+$ recombinants (compared to the number of donor bacteria in the mating). Most of the *gal*$^+$*trp*$^+$ isolates were male, and some were also *his*$^+$. These isolates were temperature-sensitive for *gal*$^+$, and segregated *his*$^-$ and *pro*$^-$ colonies on subculture at 34 C. The female isolates were temperature-stable for *gal*$^+$, *his*$^+$, and *pro*$^+$.

From these experiments, we conclude that chromosomal markers both clockwise and anticlockwise from the site of integration of the F'$_{ts}$-*gal*$^+$ element can be included in the structure of the enlarged episome. In the case of the grossly enlarged episomes seen in the PB15 by AB3511 mating, markers such as *trp*$^+$ and *his*$^+$ tend to be incorporated into the chromosomal structure of the recipient with high frequency, since, in the majority of the isolates examined, they are heat-stable and resistant to acridine orange curing from the time that they are first isolated.

Effect of introducing recA1 into donor or recipient. The data presented so far suggest that, in a proportion of the donor cells of a culture of PB15, the integrated sex factor is excised during conjugation and transferred to the recipient along with adjacent chromosomal material. To test whether the abnormal excision process is dependent on the *recA*$^+$ gene, the *recA1* allele was introduced into Hfr strain PB15 and into F$^-$ strain PB68 in the following manner. Each of these strains was used as a recipient in a mating with Hfr strain KL163, which transfers *recA1* as an early marker closely linked to *nal* (conferring resistance to nalidixic acid). In each cross, nalidixic-resistant recombinants were isolated and examined for the presence of *recA1* by testing their sensitivity to ultraviolet light. Two new strains were isolated: PB354, the *recA1* derivative of PB15; and PB349, the *recA1* derivative of PB68.

Portions of a culture of F$^-$ strain PB68 were mated with donors PB15 (Rec$^+$) and PB354 (Rec$^-$). The frequencies and genetic constitutions of the selected recombinant classes in the two crosses were virtually indistinguishable (Table 6), and resemble closely the data reported in Tables 2 and 3. Both the Rec$^+$ and the Rec$^-$ derivatives of PB15 produced *gal*$^+$ recombinants that were unstable for chromosomal markers such as *pro*$^+$, *thr*$^+$-*leu*$^+$, and *xyl*$^+$. Thus, the abnormal excision process in the donor is not dependent on the *recA*$^+$ gene.

In contrast, the presence of the *recA1* allele in the recipient had a profound effect on the frequencies, genetic constitutions, and stabilities of the various selected recombinant classes. When the Rec$^-$ strain, PB349, was used as recipient, the yield of selected chromosomal recombinants was three to four orders of magnitude lower than in parallel crosses with the Rec$^+$ recipient, PB68. Furthermore, 80 to 100% of these rare chromosomal recombinants carried a temperature-sensitive F-*gal*, in contrast to 1 to 3% for similar recombinants formed in the control cross with PB68 (Rec$^+$) as recipient. A model to account for these results will be presented in the Discussion.

In a third set of crosses (PB354 × PB349), both donor and recipient were Rec$^-$. The re-

TABLE 6. *Recombinant frequencies and unselected marker analysis of recombinants from matings between PB15 and PB68 and their recA1 derivatives*[a]

Donor[b]	Recipient[b]	Selected marker	Recombinant frequency (%)	Percentage of recombinants which inherited				
				pro$^+$	*thr*$^+$-*leu*$^+$	*xyl*$^+$	*gal*$^+$	δ
PB15 (+)	PB68 (+)	*pro*$^+$	12.8	100	16	0	2	1
PB15 (+)	PB349 (−)		4×10^{-3}	100	0.5	0	78	80
PB354 (−)	PB68 (+)		13.9	100	22.5	0	1	0
PB354 (−)	PB349 (−)		5×10^{-5}	100	0.5	0	100	100
PB15 (+)	PB68 (+)	*thr*$^+$-*leu*$^+$	6.4	46	100	0	3	3
PB15 (+)	PB349 (−)		3×10^{-4}	100	100	0	100	100
PB354 (−)	PB68 (+)		6.8	56	100	0	0	0
PB354 (−)	PB349 (−)		2×10^{-4}	—[c]	—[c]	—[c]	—[c]	—[c]
PB15 (+)	PB68 (+)	*xyl*$^+$	0.98	37	37	100	1	0.5
PB15 (+)	PB349 (−)		4×10^{-5}	—[c]	—[c]	—[c]	—[c]	—[c]
PB354 (−)	PB68 (+)		1.14	44	25	100	1	0
PB354 (−)	PB349 (−)		5×10^{-5}	—[c]	—[c]	—[c]	—[c]	—[c]
PB15 (+)	PB68 (+)	*gal*$^+$	1.2	19	9	0	100	81
PB15 (+)	PB349 (−)		0.87	10	0	0	100	100
PB354 (−)	PB68 (+)		0.50	20	10	0	100	77
PB354 (−)	PB349 (−)		6×10^{-3}	13	0	0	100	100

[a] Recombinant frequencies are given as percentages of the number of male cells in the mating mixtures. The results in this table are the means from two identical sets of experiments.

[b] The notation (+) indicates Rec$^+$; (−) indicates Rec$^-$.

[c] Insufficient numbers of recombinants for analysis.

sults were similar to those obtained with PB15 (Rec$^+$) × PB349 (Rec$^-$), with respect to the coinheritance and stability of the unselected markers. However, the absolute frequencies of selected *pro*$^+$ and *gal*$^+$ recombinants were 100-fold lower in the Rec$^-$ × Rec$^-$ cross; we have no explanation to offer for this observation.

We tested 20 male recombinants of the genetic constitution *gal*$^+$-*pro*$^-$-*thr*$^-$*leu*$^-$-*xyl*$^-$ from matings between PB349 (Rec$^-$) and the two donor strains, PB15 and PB354, for temperature sensitivity of the *gal*$^+$ and maleness markers. As anticipated, all were temperature-sensitive, but we were surprised to find that all isolates gave *gal*$^+$ sectors or papillae at 42 C. Some of the *gal*$^+$ sectors were purified and were found to be male, to be acridine orange-resistant, and to behave like Hfr donors. The frequency of integration of the *gal*$^+$ marker was about half that found when PB68 (Rec$^+$) was the recipient strain. Apparently, F-*gal*$^+$ is able to integrate into the chromosome by a mechanism not involving the *recA*$^+$ allele.

Excision of F_{ts}-gal$^+$ does not occur during normal growth of PB15. One explanation of the level of *gal*$^+$ transfer seen in matings with PB15 is that, during any 60-min period, F8-1 becomes reversibly detached in about 2% of the cells in the culture. Such detachment would be revealed on mating with a suitable *gal*$^-$ recipient, but would be masked during normal vegetative growth, the detached F-genote either becoming reintegrated or, if lost, reinfected from other cells in the culture. Thus, we reasoned that if cells were continuously cultured at densities sufficiently low so as to prevent reinfection and then plated out at 42 C, any cells that possessed a detached F-genote would be cured and thus would appear as Gal$^-$ female segregants.

PB15 was continuously cultured at less than 10^5 cells/ml in L broth at 34, 37, and 42 C. After nine generations at 37 C, the frequency of Gal$^-$ cells was 0.05%; at 30 C, it was 0.04% and at 42 C it was 0.10%. These frequencies are 20-fold to 100-fold lower than necessary to account for the excision of F_{ts}-*gal*$^+$ seen in conjugation experiments.

Although we may conclude from the experiments reported that F8-1 does not excise during vegetative growth, an alternative interpretation of the above data is to suppose that, when the F-genote excises, it removes genes adjacent to the site of integration that were not part of the original F_{ts}-*gal*$^+$. Thus, genes that are essential for growth (for example, *bio*$^+$) would become part of the temperature-sensitive replicon. Cells in which such an exci-

sion event occurred should become temperature-sensitive for growth, at least on minimal medium. We tested this suggestion by growing PB15 and spreading cells on minimal agar at 30 C. After 24 hr of growth, the colonies were replicated to minimal plates and incubated at 42 C. We tested 10,000 colonies in this way; none was found to be temperature-sensitive for growth at 42 C. We conclude from these experiments that PB15 does not excise its integrated F-genote during vegetative growth at a level sufficiently high to account for the frequency of transfer of F-*gal*$^+$ on mating.

DISCUSSION

In strain PB15, the temperature-sensitive F-*gal*$^+$ (F8-1) has undergone an unusually stable integration into the bacterial chromosome, comparable to that reported by Jacob, Brenner, and Cuzin (12) for a temperature-sensitive F-*lac*$^+$. Such stable integration is prevented in normal cells by rapid and reversible crossing-over in the region of gene duplication (3). We have carried out extensive experiments to determine why such crossing-over appears not to occur in PB15; these experiments, together with a new hypothesis to explain stabilized episome integration, will be described elsewhere (Proc. Nat. Acad. Sci. U.S.A., *in press*).

Upon mating with an F$^-$ strain, most cells in a PB15 culture behave as Hfr donors, transferring the chromosome as shown in Fig. 1. From 2 to 5% of the cells, however, behave in an anomalous fashion: they excise a segment of the chromosome which includes the integrated F_{ts}-*gal*$^+$, and transfer the resulting enlarged F-genotes to their mating partners. Such excision is analogous to the generation of F-genotes by ordinary Hfr cells, but differs from it in two respects: it occurs only during conjugation, and at an abnormally high rate (up to 5% of the cells, compared with 10^{-4} to 10^{-6} for ordinary Hfr cells) (13).

The excision process in PB15 may also be compared with the process of spontaneous deletion. Franklin (9) was able to measure the rate of spontaneous deletion in Rec$^+$ and Rec$^-$ cells by taking advantage of the fact that many mutations conferring resistance to phage T1 are due to deletions of the *tonB-trp* region (5, 18). The rate of spontaneous deletion was found to be as high in Rec$^-$ as in Rec$^+$ cells; we have shown that F_{ts}-*gal*$^+$ excision is similarly *recA*$^+$-independent in PB15.

We suggest the following hypothesis to account for the abnormal excision process in PB15. Following the proposal of Jacob, Brenner, and Cuzin (12), chromosomal replication during transfer is postulated to be controlled by action of the F-initiator on the integrated F-replicator. For the sake of our hypothesis, we assume that the initiator protein is a sequence-specific nuclease, which produces a single-strand break ("nick") at the replicator, converting the circular DNA structure into a linear one for transfer. F8-1 is proposed to carry a mutation of the F-initiator protein (the temperature-sensitive mutation or a closely linked second one), such that its specificity is altered: during conjugation it nicks not only the F-replicator, but also other sites on the chromosome, leading to the excision and transfer of chromosomal segments. Many, but not all, of these segments have one terminus in the F-replicator site.

In studies on spontaneous deletion, such as those of Franklin (9), the fate of the excised segments cannot be followed, but the broken chromosome can be inferred to have recircularized. In PB15, excised segments which include F-*gal*$^+$ are transferred and replicated normally, suggesting that they have also been circularized. We have attempted to detect circles of the predicted size in the recipients of the transferred segment, but without success. However, the isolation methods currently available are not likely to preserve circles of such a size.

The enlarged F-genotes undergo further deletions after their establishment in the recipient cells. It is possible that these deletions arise by the same mechanism which underlies abnormal excisions in PB15 (the F-initiator mutation, according to the model discussed above). In Rec$^+$ recipients, the deleted chromosomal segments become integrated into the chromosome with a high probability, resulting in recombinants which contain stable donor markers while retaining a temperature-sensitive F-*gal*$^+$. In Rec$^-$ recipients, the deletions occur at a rate which is equal to (or perhaps higher than) the rate observed in Rec$^+$ recipients; the deleted fragments are irreversibly lost, however, since their rescue by integration depends on a functioning *recA*$^+$ allele. Some integration of the F_{ts}-*gal*$^+$ does occur in the Rec$^-$ recipients, indicating a RecA$^+$-independent mechanism for F-genote (as opposed to chromosomal fragment) integration. The rare integration of F-*lac* into the chromosome of a Rec$^-$ cell has similarly been reported by DeVries and Maas (8).

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