EPISOMIC ELEMENT IN A STRAIN OF *SALMONELLA TYPHOSA*

STANLEY FALKOW AND L. S. BARON

Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Washington, D.C.

Received for publication April 27, 1962

ABSTRACT

FALKOW, STANLEY (Walter Reed Army Institute of Research, Washington, D.C.) AND L. S. BARON. Episomic element in a strain of *Salmonella typhosa*. J. Bacteriol. **84**:581-589. 1962.—An episomic element, $F_0\text{-}lac^+$, has been identified in a strain of *Salmonella typhosa* isolated from a natural habitat. The $F_0\text{-}lac^+$ element is transferred at high frequency as a single unit of transmission and replication without linkage to any other genetic character. Cells receiving $F_0\text{-}lac^+$ are heterogenotes as if $F_0\text{-}lac^+$ is not integrated as part of the linear structure of the chromosome, but rather replicates autonomously or in some other association with the genome. Evidence from complementation tests and transduction experiments is presented that the *lac* genes carried by F_0 are identical or at least markedly similar to the *lac* genes of *Escherichia coli* K-12. The F_0 transmission factor cannot be precisely identified but it does not appear to be phage or colicin. F_0 does exhibit mutual repression with the sex factor, F, of *E. coli*, and immunological experiments indicate some relationship between F and F_0 .

The term episome (Jacob and Wollman, 1958a) has been proposed for a class of genetic elements found in microorganisms. These include the sex factor, F, of bacteria, bacteriocins, and the genetic material of temperate bacteriophages, all of which differ in several respects from the usual genetic units of the bacterial chromosome. Episomes are nonessential genetic elements, since they may be absent from a cell. When absent, they may be acquired only from an external source by conjugation or transduction. When present, they may be either autonomous or fixed to the genome (Jacob, Schaeffer, and Wollman, 1960).

The properties conferred on the host by an episome depend, in part, upon the state of the

element, e.g., whether it is autonomous or integrated. In the case of the sex factor, F may be unlinked to the genome and transferred with high efficiency during conjugation (F^+ donors) (Lederberg, Cavalli, and Lederberg, 1952). In the integrated state, F replicates as part of the bacterial genome and determines the orientation of genetic material transferred during conjugation (Hfr donors; Jacob and Wollman, 1958b). Moreover, when in the integrated state, the sex factor may undergo genetic recombination with an adjacent segment of the bacterial chromosome, thereby giving rise to a new single unit of replication which may be subsequently transferred with high efficiency upon conjugation (Jacob and Adelberg, 1959; Adelberg and Burns, 1960). This process, analogous to transduction by bacteriophage, has been termed F-duction or sexduction (Jacob et al., 1960).

A donor strain of *Salmonella typhosa*, ST-2, capable of transmitting lactose utilization (lac^+) to many members of the *Enterobacteriaceae* was recently observed in this laboratory (Baron, Carey, and Spilman, 1959a). At that time, this strain was considered to be an Hfr donor. In the present communication, we report further studies with strain ST-2 and present evidence that this organism harbors an episome which has incorporated the lac^+ genetic determinants.

MATERIALS AND METHODS

Bacterial strains. Strain ST-2 is a culture of *S. typhosa* possessing the antigenic formula Vi, 9, 12:d. The fermentation pattern is like that of other strains of typhoid, with the exception that ST-2 is able to ferment lactose. This strain was isolated from the stools of a patient ill with gastroenteritis; the strain was forwarded to the Department of Bacteriology, Walter Reed Army Institute of Research. Because of its similarity to other lactose-fermenting strains of *S. typhosa* isolated in this laboratory by conjugation with *Escherichia coli* K-12 (Baron, Carey, and Spil-

man, 1959b; Baron, Spilman, and Carey, 1960), it was considered as a possible example of in vivo hybridization and was submitted to us. During our examination of this strain, it was found to transfer *lac*⁺ to many species and strains of the *Enterobacteriaceae*.

The strains of *Salmonella*, *Shigella*, and *Vibrio comma* used in this study were obtained from the culture collection of this institution. The derivatives of *E. coli* K-12 were obtained from J. Lederberg, P. D. Skaar, and S. E. Luria.

Strains of *Serratia marcescens* were received from G. Bertani or isolated from clinical material.

Bacteriophages. The Vi typing phages (Baron, Formal, and Spilman, 1955), phage PLT-22 (Zinder and Lederberg, 1952) and Plkc (Lennox, 1955), were used for transduction experiments. Phage media and assay procedures were those described by Adams (1959).

Media. Meat extract agar, minimal agar, Eosin Methylene Blue Agar (EMB) and other media used in this laboratory have been described previously (Baron et al., 1959b).

Mating procedure. Cultures of organisms to be mated were adjusted to a cell density ranging from 10^8 to 5×10^8 per ml, and mixed in appropriate volume ratios. Generally, 1 ml of the donor culture was mixed with 9 ml of the recipient culture and incubated at 37 C. Mating mixtures were diluted and plated on selective media after incubation for 90 min, unless otherwise stated.

Interruption of mating. To follow the kinetics of mating, periodic samples of a mating mixture were treated in a high-speed blender, diluted, and plated on selective media (Wollman and Jacob, 1955).

β -Galactosidase production. β -Galactosidase was measured by the hydrolysis of *o*-nitrophenyl- β -D-galactoside, using the procedures described by Pardee, Jacob, and Monod (1959). The *lac* region, as described by Jacob and Monod (1961), consists of: the *i* locus (repressor gene); the *o* locus (operator gene); the *z* locus (β -galactosidase), and the *y* locus (permease and transacetylase).

Strains were scored as *lac*⁻ if they were *z*⁻ (no β -galactosidase), *y*⁻ (no permease), or *o*⁰ (normal but nonfunctional *z* and *y* genes). The *z*⁺ strains were scored as *i*⁻ (constitutive) or *i*⁺ (inducible), with regard to enzyme synthesis,

and as *y*⁺ or *y*⁻ by induction tests with various levels of methyl- β -D-thiogalactoside (TMG).

Ultraviolet irradiation. Cultures to be irradiated were grown from 6 to 8 hr at 37 C, with aeration, in Penassay Broth (Difco); 10 ml of each culture were centrifuged, washed in buffered saline, and resuspended to the original volume in saline. The cells were irradiated in petri dishes, using constant agitation during the exposure. The source of ultraviolet light was a General Electric germicidal lamp, which was standardized against coliphage T₂ to give a dose of 10 ergs per mm² per sec at 50 cm.

Results

The ability of strain ST-2 to transfer *lac*⁺ to a variety of organisms is shown in Table 1. The results observed with *E. coli* K-12 Hfr (Cavalli), which displays a high frequency of recombination for *lac*⁺, and the same recipient organisms are also shown for comparative purposes. Strain ST-2 was promiscuous, *lac*⁺ hybrids occurring with little regard to the taxonomic status and mating polarity of the recipient cultures; *lac*⁺ was transferred with high efficiency (10^{-2} to 10^{-3} per ST-2 cell) to cultures of *Escherichia*, *Shigella*, and some *Salmonella*. Transfer to such diverse species as *Serratia marcescens* and *V. comma* was also accomplished, although with lowered efficiency (Falkow et al., 1961; Baron and Falkow, 1961). Members of the *Proteus* group and *Pseudomonas aeruginosa*, however, did not yield *lac*⁺ hybrids when grown in mixed culture with strain ST-2.

Strain ST-2 transferred *lac*⁺ to Hfr derivatives of *E. coli* and *S. abony* at frequencies slightly lower (10^{-4} to 10^{-5}) than those observed with F⁻ strains. The frequency of *lac*⁺ transfer was lowest (10^{-5} to 10^{-6}) to F⁺ cultures. *Salmonella* cultures which have been assigned the mating polarity F^o because of their initial low fertility with *E. coli* Hfr (Baron et al., 1959b) nevertheless acted as high-frequency recipients of *lac*⁺ from strain ST-2.

Transfer of donor ability. The *lac*⁺ hybrids purified from matings with ST-2 were tested with *lac*⁻ strains for their ability to transfer *lac*⁺. All hybrids tested proved to be effective donors for this marker. Generally *Escherichia*, *Salmonella*, and *Shigella* hybrids transferred *lac*⁺ with the same efficiency as did strain ST-2.

TABLE 1. Representative crosses of strains with ST-2 and *Escherichia coli* Hfr*

Recipient organism (<i>lac</i> ⁻)	Frequency of <i>lac</i> ⁺ transfer with:		
	Mating polarity	<i>E. coli</i> Hfr (Cavalli)	ST-2
<i>Salmonella typhimurium</i> TM-9	F ⁰	4 × 10 ⁻⁸	2 × 10 ⁻³
<i>S. typhimurium</i> TM-9 Sr-2	F ⁻	1 × 10 ⁻⁴	2 × 10 ⁻³
<i>S. typhosa</i> 643	F ⁰	10 ⁻⁸	5 × 10 ⁻⁴
<i>S. newport</i> ETS-50	F ⁰	10 ⁻⁸	1 × 10 ⁻⁴
<i>S. abony</i> 1446	Hfr-F ⁺	—	1 × 10 ⁻⁵
<i>Shigella dysenteriae</i> -SH	F ⁻	2 × 10 ⁻⁴	1 × 10 ⁻³
<i>S. flexneri</i> -2457T	F ⁻	1 × 10 ⁻⁴	5 × 10 ⁻⁴
<i>Escherichia coli</i> K-12 200	F ⁻	1 × 10 ⁻³	3 × 10 ⁻²
<i>E. coli</i> K-12-58-161 (<i>lac</i> ⁻)	F ⁺	1 × 10 ⁻⁷	1 × 10 ⁻⁵
<i>E. coli</i> K-12-W1895 (<i>lac</i> ⁻)	Hfr	—	1 × 10 ⁻⁴
<i>Proteus mirabilis</i> Vet 3	?	0	0
<i>Serratia marcescens</i> SM-6	?	0	4 × 10 ⁻⁸
<i>S. marcescens</i> SM-6 Sr-11	F ⁻ (?)	0	1 × 10 ⁻⁵
<i>Vibrio comma</i> 20A10	?	0	2 × 10 ⁻⁴
<i>Pseudomonas aeruginosa</i> -BH	?	0	0

* The indicated crosses were carried out under standard conditions for 120 min as described in Materials and Methods. Mating suspensions were diluted and plated on lactose medium selective for hybrids. Hfr Cavalli was contraselected by omitting methionine from the selective media; strain ST-2 was contraselected by omitting cystine and tryptophan from the selective media. The frequency of transfer is expressed as the number of *lac*⁺ hybrids recovered per donor cell in the mating suspension.

Serratia and *Vibrio* hybrids, however, varied considerably in their donor ability, ranging from 10⁻⁵ to 10⁻⁸ per cell.

Since the sex factor, F, can be transferred at high frequency to F⁻ strains without the necessity of chromosomal transfer (Lederberg et al., 1952), several attempts were made to transfer donor ability from strain ST-2 to F⁻ cells by mixed cultivation. ST-2 cells (10⁶/ml) were mixed with *E. coli* F⁻*lac*⁻ cells (10⁴/ml) in

Penassay Broth and incubated overnight. The mixture was diluted, plated on EMB lactose medium, and 100 *E. coli* colonies (still *lac*⁻) were picked and purified. None of these colonies proved to be genetic donors for *TL*⁺ (synthesis of threonine and leucine) or *gal*⁺ (galactose utilization) when crossed with suitably marked F⁻ strains. In control experiments using *E. coli* F⁺ cells in place of strain ST-2, over 70% of the clones tested were genetic donors for these same determinants. Moreover, the elimination of donor ability from strain ST-2 by treatment with acridine orange, under the conditions specified by Hirota (1960) for the elimination of the F agent, could not be accomplished.

Kinetics of *lac*⁺ transfer. By use of the technique of interrupted mating, it was found that *lac*⁺ and donor ability were transferred simultaneously within 5 min after strain ST-2 and recipient populations were mixed (Fig. 1). Since

FIG. 1. Kinetics of transfer of *lac*⁺ and donor ability by strain ST-2 to *lac*⁻ *E. coli* K-12. Conjugation interrupted by high-speed blending for 2 min, followed by dilution and plating onto selective media. Donor ability was scored among hybrids by replica plating onto plates on which about 2 × 10⁶ *lac*⁻ F⁻ cells had been spread on the surface of a selective medium.

donor ability and *lac*⁺ cannot be separated, either by conjugation or blending, it appears that they behave as a single unit of transmission and replication. We have termed this unit *F*₀-*lac*⁺. [The term *F*⁰ has been used by Baron et al. (1959b) to denote organisms which are sterile, being neither genetic recipients nor donors. In the present communication, *F*₀-*lac* describes a transmission element, *F*₀, carrying the *lac* region, the presence of which does *not* confer chromosomal donor ability nor affect the pre-existing mating polarity of the cell; i.e., cells harboring *F*₀-*lac* are not changed in their donor or recipient ability with regard to chromosomal markers.]

Transfer of markers other than lac⁺. The transfer by ST-2 and other *F*₀-*lac*⁺ cultures of a number of nutritional and fermentation determinants was tested by both selected and unselected markers in matings with mutant strains of *E. coli* K-12 and *S. typhimurium*. There was no evidence of transfer (<10⁻⁸) for the closest known markers to *lac*⁺, *ad*⁺ (synthesis of adenine), or *pro*⁺ (synthesis of proline), nor for *TL*⁺, *gal*⁺, *xyl*⁺ (utilization of xylose), or *his*⁺ (synthesis of histidine). Two other markers, closely linked to *lac*⁺, *T*_s^s (sensitivity to phage T₆) and *P*⁺ (synthesis of phosphatase), could not be detected as unselected markers.

Growth of *F*₀-*lac*⁺ strains in mixed culture with *F*⁻ strains of several species for extended periods (24 to 48 hr) also failed to reveal any evidence of gene transfer. Cells which have newly (< five generations) acquired the *F*₀-*lac*⁺ unit were similarly unable to transfer their chromosomal elements.

*Variation of F*₀-*lac*⁺ and the functional analysis of the *lac* region. Strain ST-2 and other *F*₀-*lac*⁺ organisms segregate out cells of the *lac*⁻ phenotype at a rate of 1 per 10⁵ divisions. These strains, therefore, appear to be diploid heterozygotes (heterogenotes) for the *lac* region. A large number (30 to 60%) of these *lac*⁻ segregants revert to *lac*⁺ at low frequency when incubated on EMB lactose media for several days. By appropriate crosses of these *lac*⁻ segregants with *lac*⁻ mutants of known genotype, it has been possible to demonstrate that such segregants carry a transmissible *F*₀-*lac*⁻ element (see below). These *F*₀-*lac*⁻ elements apparently arise by recombination of *F*₀-*lac*⁺ and the *lac* region of the chromosome, resulting in *lac*⁻ homogenotes, analogous to the *gal*⁻ homogenotes observed in the λ dg system

(Lederberg, 1960). *Lac*⁻ segregants which do not revert to *lac*⁺ on EMB media, nor transfer the *lac*⁻ determinant during matings, are apparently haploid and have lost the *F*₀-*lac*⁺ element. On one occasion, a haploid *lac*⁺, nondonor clone was isolated.

The functional relatedness of two mutations of similar phenotype may be tested by constructing diploid heterozygotes containing the two mutations in different configurations (Lewis, 1951; Benzer, 1957): the *cis* form, with both mutations in one chromosome, usually has the wild phenotype, while the *trans* form contains one of the mutations in each chromosome and may or may not produce the wild phenotype. If the *trans* form does produce the wild phenotype, it is concluded that the two mutations in question are located in separate functional units called *cistrons*. The availability of *F*₀-*lac*⁻ elements made it possible to apply the *cis-trans* test for complementarity to the *lac* region. In applying this test, a drop of *F*₀-*lac*⁻, streptomycin-sensitive cells (10⁸/ml) was placed on lawns of streptomycin-resistant *lac*⁻ *E. coli* K-12 derivatives on EMB lactose medium containing 600 μg/ml of streptomycin. Complementary mutations resulted in dense *lac*⁺ spots, while noncomplementary mutants usually gave rise to colorless spots or only a rare *lac*⁺ colony.

The results of these tests (Table 2) demonstrated that the *lac*⁻ mutations fell into three distinct groups. Organisms carrying a *z*⁻ mutation (no β-galactosidase) were complemented by *y*⁻ (no permease) mutants but not any other *z*⁻ mutation. The second group, organisms carry-

TABLE 2. Functional analysis of the *lac* region*

<i>F</i> ₀ - <i>lac</i> Genotype	Genotype of recipient strain			
	<i>z</i> ⁻ <i>y</i> ⁺	<i>z</i> ⁺ <i>y</i> ⁻	<i>z</i> ⁺ <i>o</i> ^h <i>y</i> ⁺	<i>z</i> ⁻ <i>y</i> ⁻
<i>F</i> ₀ <i>z</i> ⁺ <i>y</i> ⁻	+	-	-	-
<i>F</i> ₀ <i>z</i> ⁻ <i>y</i> ⁺	-	+	-	-
<i>F</i> ₀ <i>z</i> ⁺ <i>y</i> ⁺	+	+	+	+

* *F*₀-*lac*⁻ streptomycin-sensitive homogenotes were spotted on plates of Eosin Methylene Blue-lactose containing streptomycin previously seeded with 10⁸ *F*₀-*lac*⁻ bacteria of known genotype; + indicates that the mutants were complementary, producing dense hybrid patches; - indicates that the mutants were noncomplementary and produced a colorless spot or only an occasional *lac*⁺ colony.

ing a y^- mutation, were complemented by z^- mutants but not any other y^- mutant. Finally, one mutant strain carrying an operator mutation, o^0 (normal but nonfunctional z and y genes) was not complemented by any z^- or y^- mutation tested, and presumably the o gene belongs to a *cistron* distinct from z and y . These findings are in accord with those observed by Jacob and Monod (1961) on much more extensive material in *E. coli* K-12.

β -Galactosidase production. F_0 - lac^+ cultures grown in a synthetic medium with glycerol as the sole carbon source and induced with 2×10^{-3} M TMG produced roughly 2.5 times as much β -galactosidase as did haploid *E. coli* K-12 cells. This increased synthesis of β -galactosidase produced by F_0 - lac^+ strains may indicate the presence of more than one F_0 - lac^+ element per cell. In the absence of inducer, F_0 - lac^+ cells synthesized approximately 10 to 30% of the fully induced level of β -galactosidase. Strain ST-2 and other F_0 - lac^+ cultures are, therefore, partially constitutive for β -galactosidase.

Previous studies (Pardee et al., 1959; Jacob and Monod, 1961) have clearly shown that the inducible i^+ allele is dominant over the constitutive i^- , and that it is active in both the *cis* and *trans* positions with respect to the z and y genes.

TABLE 3. β -Galactosidase synthesis by haploids and diploids of different genotype*

Genotype	β -Galactosidase activity in arbitrary units	
	Noninduced	Induced
$i^+o^+z^+y^+/-$	<1	100
$i^+o^+z^+y^-/-$	<1	85
$i^-o^+z^+y^+/-$	95	95
$i^+o^0z^+y^+/-$	<1	1
$i^+o^+z^-y^+/-$	<1	1
$-/F_0z^+y^+$	51	201
$i^+o^+z^+y^-/F_0z^+y^+$	37	217
$i^+o^+z^+y^-/F_0z^-y^+$	<1	83
$i^+o^+z^-y^+/F_0z^+y^-$	70	235
$i^-o^+z^+y^-/F_0z^+y^+$	51	280
$i^-o^+z^+y^-/F_0z^-y^+$	<1	98
$i^+o^0z^+y^+/F_0z^+y^+$	22	234

* Cells were grown in synthetic medium with glycerol as carbon source, with or without 10^{-3} M methyl- β -D-thiogalactoside. Values of β -galactosidase are given as percentages of induced wild type *E. coli* K-12.

TABLE 4. Effect of ultraviolet irradiation on the transfer of F_0 - lac^{++}

Time incubated after irradiation	No. of lac^+ hybrids/ 10^6 viable cells
<i>min</i>	
0 (before irradiation)	6.4×10^3
0 (after irradiation)	6.8×10^3
30	9.5×10^3
45	3.2×10^4
60	7.4×10^4
90	9.5×10^4
120	1.2×10^5
150	7.2×10^3

* ST-2 cells were irradiated to give 30% survivors, centrifuged, and resuspended in broth at 37 C. At intervals, samples were withdrawn, assayed, added to unirradiated F^- *E. coli* lac^- cells, and plated immediately on selective media.

By use of suitable strains, lac^+ diploid heterozygotes of the constitution $i^+z^-y^+/F_0z^+y^+$ and $i^-z^+y^-/F_0z^-y^+$ were prepared. In the first instance, the diploid remained partially constitutive even though the i^+ allele was present on the chromosome. In the second instance, the diploid behaved phenotypically as i^+ despite the i^- nature of the chromosomal segment. From these experiments and others, as shown in Table 3, it appears that the F_0 - lac^+ element carries the i^+ allele but contains some other mutation responsible for the partial constitutive synthesis in F_0 - lac^+ strains. Such a mutation has been recently described (Jacob et al., 1960), in which the operator gene, o , has mutated to the dominant constitutive form, o^c . In line with the data of Table 3, an o^c mutation affects only the structural genes which are *cis* but not those in the *trans* position. At present we feel that the genotype of the F_0 - lac^+ element is $i^+o^cz^+y^+$.

Effect of ultraviolet irradiation on F_0 - lac^+ . When strain ST-2 was exposed to ultraviolet irradiation (UV) and then mated with a suitable nonirradiated recipient, the cells lost their ability to transfer F_0 - lac^+ exponentially as a function of the UV dose. On the other hand, if irradiated ST-2 cells were briefly incubated in broth, and periodic samples were assayed for F_0 - lac^+ transfer, there was a 10- to 50-fold increase in transfer per viable cell above comparable nonirradiated controls (Table 4). Increased transfer was first evident after 30 min of incubation, reached a

maximum after 90 to 120 min of incubation, and had declined by 150 min of incubation.

While the conditions necessary for the enhancement of $F_0\text{-}lac^+$ transfer proved similar to those described previously for the induction of prophage maturation and the induced biosynthesis of bacteriocins (Jacob, Siminovitch, and Wollman, 1953), lysis or death of the ST-2 cells was not observed, as evidenced by a relatively constant viable-cell count during the incubation period. It is not yet clear whether the effect of UV is to increase the actual number of $F_0\text{-}lac^+$ units or to increase the efficiency of transfer.

Transduction of the $F_0\text{-}lac^+$ element. Transduction of $F_0\text{-}lac^+$ by the Vi phages, PLT-22, and Plkc was attempted. In the case of the Vi and PLT-22 phages, transduction was not observed ($1 < 0^{-9}$ per absorbed phage).

Transduction of lac^+ by phage Plkc was demonstrated at a frequency of 10^{-5} to 10^{-6} per absorbed phage to lac^- *E. coli* K-12 derivatives, using lysates prepared from *E. coli* $F_0\text{-}lac^+$ strains. These transductant clones were stable lac^+ , and less than 1% were able to transfer lac^+ by conjugation to lac^- cultures.

Transduction of lac^+ from *E. coli* $F_0\text{-}lac^+$ to *S. dysenteriae* Sh and *S. flexneri* 2a-120 was observed at a frequency of 10^{-7} to 10^{-8} per absorbed phage. These lac^+ transductants were more or less unstable, but relatively stable subclones were readily isolated. Generally, these clones were Pl immune (Pli) but yielded only a few active phage particles or none. Roughly 10% were Pl sensitive. Thirty of these clones were selected and tested for the transfer of lac^+ by conjugation to *Salmonella*. Nine of them transferred lac^+ at a frequency of approximately 10^{-6} per donor cell. The lac^+ *Salmonella* progeny were capable of transferring lac^+ serially and displayed the characteristics of $F_0\text{-}lac^+$ cultures. Preliminary results involving transfer of lac^+ from some of the

Shigella transductants to derivatives of *E. coli* K-12 resulted in a few lac^+ Pli progeny. The lac^+ Pli property could be transmitted serially and may be the result of a new $F_0\text{-}lac^+$ -Pl element. These transduction results are summarized in Table 5.

The differences observed between transduction of lac^+ from *E. coli* to *E. coli* and *E. coli* to *S. dysenteriae* are apparently the result of differences in homology between the phage-carried lac^+ and the recipient chromosome. In the former case, the lac^+ marker is probably readily integrated into the recipient chromosome, while in the latter, transduction by lysogenization, is the more probable outcome (Luria, Adams, and Ting, 1960; Franklin and Luria, 1961).

Nature of F_0 . Heat-killed cells, sterile culture filtrates, and deoxyribonucleic acid derived from strain ST-2 and other $F_0\text{-}lac^+$ cultures cannot accomplish the transfer of lac^+ to lac^- cultures. Moreover, $F_0\text{-}lac^+$ cells do not elaborate bactericidal substances analogous to colicin. The genetic element involved in the transmission of lac^+ from ST-2, therefore, does not have the properties of temperate phage or colicin, but behaves in a manner rather similar to the sex factor of bacteria.

The autonomous (F^+) state and integrated (Hfr) state of the sex factor appear to be mutually exclusive, although variants of the sex factor are known in which a rapid alteration between the two states occur (Jacob et al., 1960). As mentioned previously, $F_0\text{-}lac^+$ is transferred to cultures carrying both autonomous and integrated sex factor, although with lowered efficiency. An examination of F^- , F^+ , and Hfr bacteria harboring $F_0\text{-}lac^+$ discloses (Table 6) a relationship involving some mutual repression (regulation).

F^- bacteria which have received $F_0\text{-}lac^+$ are unchanged with regard to their fertility with Hfr

TABLE 5. Transduction of lac^+ from strains carrying $F_0\text{-}lac^+$

Phage	Donor strain	Recipient strain	Transduction frequency	Donor ability
Vi-C ₂	<i>S. typhosa</i> $F_0\text{-}lac^+$	<i>S. typhosa</i> lac^-	10^{-9}	
PLT-22	<i>S. typhimurium</i> $F_0\text{-}lac^+$	<i>S. typhimurium</i> LT-2	10^{-9}	
Plkc	<i>E. coli</i> K-12 $F_0\text{-}lac^+$	<i>E. coli</i> K-12 lac^-	10^{-5}	1%
Plkc	<i>E. coli</i> K-12 $F_0\text{-}lac^+$	<i>S. dysenteriae</i> Sh	10^{-7}	30%
		<i>S. flexneri</i> 2a-120		

TABLE 6. Fertility of F^+ and Hfr strains after acquisition of $F_0\text{-}lac^{+*}$

Cross	Selection for	Frequency	F^+ infection %
<i>E. coli</i> Hfr $\times$ <i>E. coli</i> F^-	Threonine and leucine	2×10^{-3}	
<i>E. coli</i> Hfr $\times$ <i>E. coli</i> F^- ($F_0\text{-}lac^+$)	Threonine and leucine	2×10^{-3}	
<i>E. coli</i> $F^+ \times E. coli$ F^-	Threonine and leucine	3×10^{-6}	85-90
<i>E. coli</i> $F^+ \times E. coli$ F^- ($F_0\text{-}lac^+$)	Threonine and leucine	3×10^{-6}	1-12
<i>E. coli</i> Hfr ($F_0\text{-}lac^+$) $\times$ <i>E. coli</i> F^-	Threonine and leucine	2×10^{-3}	
<i>E. coli</i> F^+ ($F_0\text{-}lac^+$) $\times$ <i>E. coli</i> F^-	Threonine and leucine	3×10^{-6}	85-90
<i>E. coli</i> Hfr ($F_0\text{-}lac^+$) $\times$ <i>E. coli</i> F^-	Lactose	1×10^{-5}	
<i>E. coli</i> F^+ ($F_0\text{-}lac^+$) $\times$ <i>E. coli</i> F^-	Lactose	1×10^{-5}	1
<i>E. coli</i> Hfr $\times$ <i>E. coli</i> F^-	Threonine, leucine, and lactose	8×10^{-4}	
<i>E. coli</i> Hfr ($F_0\text{-}lac^+$) $\times$ <i>E. coli</i> F^-	Threonine, leucine, and lactose	$< 10^{-6}$	

* Lac^- *E. coli* Hfr (Cavalli) and F^+ (58-161) cultures were obtained by UV treatment and were infected with $F_0\text{-}lac^+$. The specified matings were carried out with an F^- lac^- TLB₁ *E. coli* stock, W1117. Both the Hfr and F^+ strain were contraselected by omitting methionine from the selective media. Frequencies are expressed as the number of recombinants isolated per donor cell in the mating mixtures.

and F^+ strains for chromosomal determinants. These strains, however, do display an increased resistance to infection by the sex factor.

$F^+(F_0\text{-}lac^+)$ clones show the usual low frequency of recombination for chromosomal genes, but transmit lac^+ at somewhat lower (10^{-5} /donor cell) efficiency than $F^-(F_0\text{-}lac^+)$ strains or ST-2. Recombinant progeny resulting from crosses with $F^+(F_0\text{-}lac^+)$ cultures are virtually all F^+ but not $F_0\text{-}lac^+$, while clones which have received $F_0\text{-}lac^+$ from the same donor population are all F^- .

Hfr ($F_0\text{-}lac^+$) strains transfer their chromosome with the same rate and orientation as those which are stable lac^+ . The transfer of $F_0\text{-}lac^+$ to zygotes occurs at a slow rate but begins very early (<5 min) and appears to be unlinked with other genetic markers. It is interesting to note that $F_0\text{-}lac^+$ transfer by Hfr, as well as by F^+ cultures, is somewhat reduced (0.1-0.01) in frequency. In some Hfr ($F_0\text{-}lac^+$) stocks, the $F_0\text{-}lac^+$ element is very unstable and segregates out haploid lac^- colonies at a frequency of 1 in 1,000 divisions. Thus, although one might suspect that the lac genes carried by F_0 would insure specific pairing with the lac region of the chromosome, these results suggest that $F_0\text{-}lac^+$ enters zygotes separately from the Hfr chromosome. The $F_0\text{-}lac^+$ element, therefore: (i) may occupy some specific extrachromosomal site which insures rapid transmission; (ii) may become de-

TABLE 7. Agglutination of F^+ , Hfr, $F_0\text{-}lac^+$, and sexduced cells in F^+ antiserum*

Antigen	Titer in F^+ antiserum
<i>E. coli</i> K-12 F^-	<5
<i>E. coli</i> K-12 F^+	640
<i>E. coli</i> K-12 Hfr	640
<i>E. coli</i> K-12 F^- ($F_0\text{-}lac^+$)	40
<i>S. typhosa</i> 643 F^-	<5
<i>S. typhosa</i> 643 F^- ($F_0\text{-}lac^+$)	20
<i>S. typhosa</i> F^+	320
<i>S. typhosa</i> F^+ 13	640
<i>S. typhosa</i> F^+ 39	160

* Agar-grown (8 hr) cells (about 10^7 organisms per ml) were used for slide agglutination in 1:5 antiserum. Cell suspensions exhibiting a positive reaction were titrated (with twofold serial dilutions 1:5 serum) in tubes left at room temperature overnight. The values refer to the reciprocal of the highest antibody dilution showing agglutination. The F^+ 13 and F^+ 39 strains of *S. typhosa* 643 were obtained by sexduction, using *E. coli* W3747 (F^+ 13 donor) obtained from J. Lederberg and *E. coli* 58-161 from which F^+ 39 was isolated.

tached from the chromosome during mating; or (iii) may undergo alteration between a chromosomal and extrachromosomal location. The last possibility is especially useful to explain the effect of ultraviolet irradiation on $F_0\text{-}lac^+$.

The mutual, although incomplete, repression

of F_0 and the sex factor led us to consider the possibility of a relationship between the two. Antiserum specific for an antigen (Orskov and Orskov, 1960) elaborated by F^+ , and Hfr, but not F^- cells was prepared and tested against strains of bacteria before and after the acquisition of F_0-lac^+ . As shown in Table 7, this serum agglutinated F^+ , Hfr, and sexduced cells to an average titer of 1:640. F_0-lac^+ cells were agglutinated weakly, but not their F^- parents.

Further studies to demonstrate a relationship between F and F_0 were performed using a phage (Loeb, 1960) which lyses only cultures carrying the sex factor. This phage, however, did not lyse or absorb to $F^-(F_0-lac^+)$ cells. $F^+(F_0-lac^+)$ and Hfr (F_0-lac^+) cells remain fully susceptible to this phage.

DISCUSSION

The unifying concepts for the agents known as episomes have been discussed in detail previously (Jacob and Wollman, 1958a; Jacob et al., 1960). They are dispensible genetic elements which, when present, may be either autonomous or integrated. The results of this study are consistent with the view that the F_0-lac^+ element meets these criteria. F_0-lac^+ is transferred serially and at high frequency as a single unit of transmission and replication without linkage to any other genetic character. Cells receiving F_0-lac^+ are heterogenotes as if F_0-lac^+ is not integrated as part of the linear structure of the chromosome, but rather replicates autonomously or in some other association with the genome. F_0-lac^+ may become integrated after transduction by phage or, rarely, by interaction with the chromosome. The F_0-lac^+ element, therefore, behaves as an episomic element in which a transmission agent, F_0 , has incorporated the *lac* locus and subsequently mutated to the autonomous state as the unit F_0-lac^+ . Since *Salmonella* organisms are *lac^-*, ST-2 probably acquired the F_0-lac^+ element by conjugation in nature, either from the original host or one of its derivatives.

The *lac* genes carried by F_0 are identical or at least very similar to the *lac* of *E. coli* K-12. F_0-lac^+ is readily transduced by phage P1 and becomes stably integrated into *E. coli* K-12. Likewise, the complementation studies with F_0-lac^+ demonstrate their close similarity. These observations indicate that the *lac* region carried by F_0 was initially located on the chromosome of a member

of the *Escherichia* group, as contrasted to other *lac^+* enteric organisms such as *Aerobacter*, which differ significantly from *Escherichia* in the base composition of their deoxyribonucleic acid (Marmur, Schildkraut, and Doty, 1961).

The transmission agent F_0 cannot be precisely identified. It does not appear to be phage or colicin, since it does not harm host cells and is transferred only by conjugation. The mutual repression of F_0 and the sex factor, as well as our immunological data, would seem to point to some relationship. On the other hand, F_0 does not confer chromosomal fertility upon recipient cells, as does the sex factor and sexduction. Indeed, F_0 may be only operationally defined because of its association with the *lac* genes.

Since F_0-lac^+ very likely originated in a member of the *Escherichia* group, it may be profitable to recall that several sex factors, related but distinct from F^+ , have been described in wild strains of *E. coli*. These strains varied considerably in their ability to infect and confer chromosomal fertility upon recipient cells. F_0 , therefore, might fail to confer chromosomal fertility because of heterogeneity or mutation. Perhaps F_0 requires an auxiliary factor for successful chromosomal transfer, analogous to the effect of colicin E_1 on colicin I in *Salmonella* (Ozeki and Howarth, 1961). It is interesting to note that another episome, RTF, also isolated from a natural habitat, was initially reported (Watanabe and Fukasawa, 1961) to be incapable of mediating chromosomal transfer. Further studies (Falkow and Ryman, unpublished data), however, indicate that RTF can mediate low-frequency gene transfer under proper experimental conditions. It is hoped that the proper conditions and hosts will be found in which to study chromosomal transfer by F_0-lac^+ strains.

ACKNOWLEDGMENTS

The authors wish to thank J. A. Wohlhieter, Julius Marmur, and Robert Rownd for their interest and suggestions. Howard T. Bausum performed many of the phage experiments. The technical assistance of I. R. Ryman is gratefully acknowledged.

LITERATURE CITED

- ADAMS, M. 1959. Bacteriophages. Interscience Publishers, New York.
- ADELBERG, E. A., AND S. N. BURNS. 1960. Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. 79:321-330.

- BARON, L. S., W. F. CAREY, AND W. M. SPILMAN. 1959a. Characteristics of a high frequency of recombination (Hfr) strain of *Salmonella typhosa* compatible with *Salmonella*, *Shigella* and *Escherichia* species. Proc. Natl. Acad. Sci. U.S. **45**:976-983.
- BARON, L. S., W. F. CAREY, AND W. M. SPILMAN. 1959b. Hybridization of *Salmonella* species by mating with *Escherichia coli*. Science **130**: 976-977.
- BARON, L. S., AND S. FALKOW. 1961. Genetic transfer of episomes from *Salmonella typhosa* to *Vibrio cholerae*. Records Genetics Soc. Am. **30**:59.
- BARON, L. S., S. B. FORMAL, AND W. M. SPILMAN. 1955. Vi phage-host interaction in *Salmonella typhosa*. J. Bacteriol. **69**:177-183.
- BARON, L. S., W. M. SPILMAN, AND W. F. CAREY. 1960. Diploid heterozygous hybrids from matings between *Escherichia coli* and *Salmonella typhosa*. J. Exptl. Med. **112**:361-372.
- BENZER, S. 1957. The elementary units of heredity, p. 70-93. In W. D. McElroy and B. Glass [ed.], The chemical basis of heredity. Johns Hopkins University Press, Baltimore.
- FALKOW, S., J. MARMUR, W. F. CAREY, W. M. SPILMAN, AND L. S. BARON. 1961. Episomic transfer between *Salmonella typhosa* and *Serratia marcescens*. Genetics **46**:703-706.
- FRANKLIN, N. C., AND S. E. LURIA. 1961. Transduction by bacteriophage ϕ 1 and the properties of the *lac* genetic region in *E. coli* and *S. dysenteriae*. Virology **15**:299-311.
- HIROTA, Y. 1960. The effect of acridine dyes on mating type factors in *E. coli*. Proc. Natl. Acad. Sci. U.S. **46**:57-64.
- JACOB, F., AND E. A. ADELBERG. 1959. Transfert de caractères génétiques par incorporation au facteur sexuel d'*Escherichia coli*. Compt. rend. **249**:189-191.
- JACOB, F., AND J. MONOD. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **3**:318-356.
- JACOB, F., D. PERRIN, C. SANCHEZ, AND J. MONOD. 1960. L'opéron: groupe de gènes à expression coordonnée par un opérateur. Compt. rend. **250**:1727-1729.
- JACOB, F., P. SCHAEFFER, AND E. L. WOLLMAN. 1960. Episomic elements in bacteria. Symposium Soc. Gen. Microbiol. **10**:67-91.
- JACOB, F., L. SIMINOVITCH, AND E. L. WOLLMAN. 1953. Comparaison entre la biosynthèse induite de la colicine et des bactériophages et entre leur mode d'action. Ann. inst. Pasteur **84**:313-318.
- JACOB, F., AND E. L. WOLLMAN. 1958a. Les episomes, éléments génétiques ajoutés. Compt. rend. **247**:154-156.
- JACOB, F., AND E. L. WOLLMAN. 1958b. Genetic and physical determinations of chromosomal segments in *E. coli*. Biological replication of macromolecules. Symposia Soc. Exptl. Biol. **12**:75-92.
- LEDERBERG, E. M. 1960. Genetic and functional aspects of galactose metabolism in *Escherichia coli* K-12. Symposium Soc. Gen. Microbiol. **10**:115-131.
- LEDERBERG, J., L. L. CAVALLI, AND E. M. LEDERBERG. 1952. Sex compatibility in *E. coli*. Genetics **37**:720-730.
- LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage ϕ 1. Virology **3**:22-41.
- LEWIS, E. B. 1951. Pseudo alleles and gene evolution. Cold Spring Harbor Symposia Quant. Biol. **16**:159-172.
- LOEB, T. 1960. The isolation of a bacteriophage specific for the F^+ and Hfr mating types of *E. coli* K-12. Science **131**:932-933.
- LURIA, S. E., J. N. ADAMS, AND R. C. TING. 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae*, and the properties of the transducing phage particles. Virology **12**:348-390.
- MARMUR, J., C. L. SCHILDKRAUT, AND P. DOTY. 1961. The reversible denaturation of DNA and its use in studies of nucleic acid homologies and the biological relatedness of microorganisms. J. chim. phys., **58**:945-955.
- ORSKOV, I., AND F. ORSKOV. 1960. An antigen termed f^+ occurring in F^+ *E. coli* strains. Acta Pathol. Microbiol. Scand. **48**:37-46.
- OZEKI, H., AND S. HOWARTH. 1961. Colicine factors as fertility factors in bacteria. Nature **190**: 986-988.
- PARDEE, A. B., F. JACOB, AND J. MONOD. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β -galactosidase by *E. coli*. J. Mol. Biol. **1**:165-178.
- WATANABE, T., AND T. FUKASAWA. 1961. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. I. Transfer of resistance factors by conjugation. J. Bacteriol. **81**:669-678.
- WOLLMAN, E. L., AND F. JACOB. 1955. Sur le mécanisme du transfert de matériel génétique au cours de la recombinaison chez *E. coli* K-12. Compt. rend. **240**:2449-2451.
- ZINDER, N. D., AND J. LEDERBERG. 1952. Genetic exchange in *Salmonella*. J. Bacteriol. **64**: 679-699.