

TRANSFER OF EPISOMIC ELEMENTS TO *PROTEUS*

I. TRANSFER OF F-LINKED CHROMOSOMAL DETERMINANTS

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ABSTRACT

FALKOW, STANLEY (Walter Reed Army Institute of Research, Washington, D.C.), J. A. WOHLHIETER, R. V. CITARELLA, AND L. S. BARON. Transfer of episomic elements to *Proteus*. I. Transfer of F-linked chromosomal determinants. *J. Bacteriol.* **87**:209-219. 1964.—F-linked *lac*⁺ genes may be transferred from *Escherichia coli* to several species of *Proteus* by conjugation. Usually the transferred genetic elements are markedly unstable in *Proteus*, but repeated plating permits the selection of relatively stable *Proteus lac*⁺ strains. *Proteus* strains carrying F-linked *lac*⁺ markers are heterogenotes and limited donors for *lac*⁺. In addition, both the fertility and *lac*⁺ property may be eliminated from *Proteus* by treatment with acridine orange. *Escherichia* and *Proteus* possess very different overall deoxyribonucleic acid (DNA) base compositions. In CsCl density gradients of DNA extracted from *Proteus lac*⁺ strains, the acquisition of *Escherichia* genes by *Proteus* may be correlated with the addition of a physically recognizable high molecular weight, native DNA fraction of *Escherichia* base composition. *Proteus lac*⁺ strains synthesize a β -galactosidase which is indistinguishable from *E. coli* enzyme by several criteria. Despite this specificity, the regulatory functions of *Escherichia lac*⁺ genes appear to be impaired in *Proteus*.

and Adelberg, 1959; Adelberg and Burns, 1960). This phenomenon has been termed F-duction or sex-duction (Jacob, Schaeffer, and Wollman, 1960).

Unlike most gene-transfer mechanisms in bacteria which only occur between closely related cell populations, it has been determined that F-duction is relatively promiscuous. Initially, Jacob and Adelberg (1959) found that F which had incorporated the lactose genes (*F-lac*) could be transmitted at a high frequency between strains of *Escherichia*, *Salmonella*, and *Shigella*. Studies from this laboratory (Falkow et al., 1961) demonstrated that *F-lac* and other episomic elements could be transferred between *Salmonella* and *Serratia marcescens*, despite the marked divergence in the guanine plus cytosine (GC) content in the deoxyribonucleic acid (DNA) of these organisms (50 and 58% GC, respectively). Further studies have described the transfer of *F-lac* across presently constituted family lines to *Vibrio comma* (Baron and Falkow, 1961) and *Pasteurella pestis* (Martin and Jacob, 1962). The transfer of other episomic elements, R-factor (Watanabe, 1963), *F_o-lac* (Falkow and Baron, 1962), and colicinogenic factors (Smith and Stocker, 1962), have also been described among many species of the Enterobacteriaceae.

Temperate phages, colicinogenic factors, and sex factors comprise a group of genetic elements, termed episomes, which can alternate between a state of stable attachment to the chromosome and a state of autonomy (Jacob and Wollman, 1958). It has been discovered that the sex factor, F, of *Escherichia coli* K-12 can incorporate chromosomal fragments adjacent to its site of attachment in Hfr bacteria. Upon reversion to the autonomous state, such F-linked chromosomal determinants (episomic elements) may be transferred with high efficiency, independently of the intact chromosome, to suitable recipient bacteria (Jacob

A possible explanation for the observation of gene transfer between organisms with divergent properties and DNA base compositions comes from an examination of fractionated DNA derived from bacterial strains harboring episomic elements. In *S. marcescens* strains infected with the *F-lac* element of *E. coli*, the DNA band profile obtained by density-gradient centrifugation shows a species of molecules not apparent in DNA derived from the *Serratia* parental strain (Marmur et al., 1961). The density of this "new" DNA band was identical to that of the *E. coli* parental strain, which suggested that it could be

attributed to the *F-lac* element and that this element was not integrated into the host genome but rather replicated autonomously.

Members of the *Proteus* group exhibit DNA base compositions which differ markedly from all other members of the Enterobacteriaceae (Falkow, Ryman, and Washington, 1962b). In the present communication, we describe the transfer to *Proteus* species of several F-linked chromosomal elements. *Proteus* strains harboring these genetic elements display a marked heterogeneity of their DNA even in unfractionated preparations.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 or *Salmonella typhosa* strains carrying the genetic elements *F-lac* (Jacob and Adelberg, 1959), *F_o-lac* (Falkow and Baron, 1962), and *F'-13* (Hirota and Sneath, 1961) were employed as donor strains. The *F-lac* and *F_o-lac* elements consist of a fertility factor and the genes governing the utilization of lactose (*lac*⁺) as the only known genetic determinants; the *F'-13* element carries, in addition to the fertility factor, the genetic information for *lac*⁺, the synthesis of alkaline phosphatase (*P*⁺), and the synthesis of adenine (*ad*⁺).

A strain of *Proteus mirabilis* labeled PM-1 served as the recipient in most genetic crosses. This strain is stably *lac*⁻ and requires nicotinic acid for growth on minimal medium. Strains of *P. rettgeri* and *P. vulgaris* were employed as recipient strains in some experiments.

Media. Meat extract-agar and minimal medium were prepared as described in a previous communication (Falkow, Rownd, and Baron, 1962a).

Mating procedure. Bacteria were individually grown in Penassay Broth to a concentration of approximately 5×10^8 cells per ml. The cultures were mixed to give about 2×10^8 cells per ml of the donor strain and 5×10^8 cells per ml of the recipient strain. Mating mixtures were incubated at 37 C for 3 hr, and then diluted and plated on selective media. Selection was performed for lactose utilization on minimal medium containing 0.3% lactose and 10 mg per liter of nicotinic acid. The lack of essential growth factors in the minimal medium served to contraselect the donor strains. Incubation of mating plates was routinely done at 37 C for 5 to 7 days.

Preparation of DNA and density-gradient centrifugation. DNA was extracted from bacterial strains by the method of Marmur (1961).

The method of CsCl density-gradient centrifugation was similar to that described by Meselson, Stahl, and Vinograd (1957). Approximately 3 to 6 μ g of DNA were added to a 57% (w/w) stock solution of CsCl (American Potash & Chemical Corp., Los Angeles, Calif.) buffered to pH 8.0 with 0.015 M tris(hydroxymethyl)aminomethane (tris) buffer, and the density of the sample was adjusted to 1.718 g/cm³ by use of the linear relationship between refractive index and density. Approximately 0.75 ml of the final CsCl solution was placed in a cell containing a plastic Kel-F centerpiece (Minnesota Mining & Manufacturing Co., St. Paul, Minn.) and centrifuged in a Spinco model E analytical ultracentrifuge at 44,770 rev/min at 25 C. After 20 hr of centrifugation, the banded DNA was photographed by use of ultraviolet-absorption optics. The photographs were traced with a Joyce-Loebl Mark IIIB double-beam recording microdensitometer, and the buoyant densities of the DNA were calculated by reference to a standard DNA of known density included in each run. Since the buoyant density of DNA is a function of composition, the GC composition of the banded DNA could be easily calculated (Schildkraut, Marmur, and Doty, 1962).

Chromatographic fractionation of DNA. Methylated-albumin-kieselguhr (MAK) columns were prepared according to Mandell and Hershey (1960). Columns 7.5 cm high and 2.5 cm wide were loaded with 0.9 to 1.5 mg of DNA. Linear NaCl concentration gradients were employed and 5-ml fractions were collected. The optical density of the fractions was determined at 260 m μ in a Beckman DU spectrophotometer, and the NaCl gradient was monitored by refractive index measurements on selected fractions.

β -Galactosidase determinations. β -Galactosidase was assayed on toluenized cell suspensions according to the procedures described by Pardee, Jacob, and Monod (1959). Isopropyl- β -D-thiogalactoside (IPTG) was employed as the inducer of enzyme activity. In several instances, sonic extracts of fully induced bacteria were prepared in a 10-kc Raytheon sonic oscillator for use in enzyme assays and as a source of enzyme for certain physical measurements to be described below.

RESULTS

The donor *F-lac*⁺ and *F_o-lac*⁺ strains transferred *lac*⁺ to *Proteus mirabilis* and *P. vulgaris* at a frequency of 10^{-4} to 10^{-6} per donor cell, while *lac*⁺ transmission by the *F'-13* donor could be

TABLE 1. Transfer of *lac*⁺ to *Proteus* species

Donor	Recipient	Frequency of transfer per donor cell
<i>Escherichia coli</i> K-12 F- <i>lac</i> ⁺	<i>P. mirabilis</i>	4×10^{-6}
	<i>P. vulgaris</i>	1×10^{-6}
	<i>P. rettgeri</i> 1	3×10^{-7}
	<i>P. rettgeri</i> 2	$<10^{-8}$
<i>E. coli</i> K-12 F'-13	<i>P. mirabilis</i>	2×10^{-7}
	<i>P. vulgaris</i>	1×10^{-8}
<i>Salmonella typhosa</i> F _o - <i>lac</i> ⁺	<i>P. mirabilis</i>	2×10^{-4}
	<i>P. vulgaris</i>	3×10^{-4}
	<i>P. rettgeri</i> 1	3×10^{-5}
	<i>P. rettgeri</i> 2	$<10^{-8}$

detected only at a frequency of 10^{-7} to 10^{-8} per donor cell (Table 1). The *P. rettgeri* strains employed accepted the *lac*⁺ genes less readily or not at all. When the *lac*⁺ *Proteus* clones appearing on mating plates were restreaked on the same medium used for their selection, large numbers of minute colonies (about 0.4 mm in diameter), and only an occasional normal-sized colony, were observed. If minute colonies were completely picked up in saline and again replated, about 10%, were capable of producing another single minute colony. With each successive restreaking, however, the proportion which yielded minute colonies was reduced by about one-third. This phenomenon is reminiscent of abortive transduction (Ozeki, 1956) and, by analogy, we assume that the minute colonies are the result of the linear inheritance of a nonreplicating persistent *lac*⁺ exogenote.

The normal-sized colonies could be maintained on selective media indefinitely, although on complete indicator medium (MacConkey agar) they appeared as mixtures of *lac*⁻ and sector or mosaic *lac*⁺ clones (Fig. 1). By repeated transfer of *lac*⁺ sectors of the latter, it was possible to obtain relatively stable *lac*⁺ *Proteus* strains from each of the genetic crosses. These strains were maintained on MacConkey agar, but, despite daily single-colony reisolutions, they have continued to segregate *lac*⁻ progeny at a frequency of about 3×10^{-3} (Table 2).

Transmission of *lac*⁺ by *Proteus*. *Proteus lac*⁺ strains from each of the genetic crosses were tested for their ability to act as genetic donors for *lac*⁺ to *lac*⁻ *E. coli* K-12 F⁻ cultures. All of the *Proteus* strains were donors for this marker, although the frequency of transfer varied consid-

erably (Table 3). Also, it should be noted that many of the *Proteus lac*⁺ clones derived from the F'-13 donor transferred the *P*⁺ genes and *ad*⁺

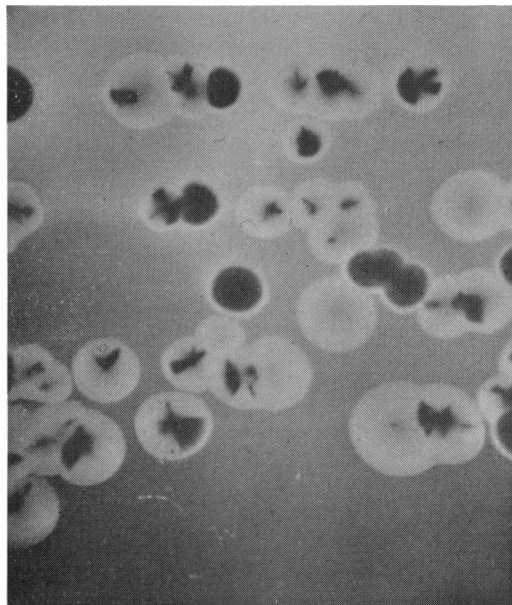


FIG. 1. Initial instability of *Proteus lac*⁺ strains. Appearance of PM-1 F'-13 hybrid strain on MacConkey agar showing mixture of *lac*⁻, mosaic *lac*⁺, and uniformly *lac*⁺ colonies. Relatively stable *lac*⁺ derivatives may be obtained from the latter.

TABLE 2. Segregation of *lac*⁻ clones from relatively stable *lac*⁺ *Proteus* strains*

Strain	Length of incubation (days)	No. of colonies examined	No. <i>lac</i> ⁺	No. <i>lac</i> ⁻	Per cent <i>lac</i> ⁻
PM-1 F _o - <i>lac</i> ⁺	1	480	477	3	0.6
	3	480	465	15	3.0
PM-1 F- <i>lac</i> ⁺	1	725	723	2	0.3
	3	746	739	7	1.0
PM-1 F'-13	1	643	641	2	0.3
	3	715	706	9	1.3

* A single *lac*⁺ colony from each strain was suspended in 1 ml of physiological saline, and approximately 50 cells were inoculated into flasks containing 50 ml of Penassay Broth. These cultures were grown to a cell density of 5×10^8 per ml, diluted, and plated on MacConkey agar to score for *lac*⁻ segregants. A 0.1-ml amount of culture was used to inoculate 50 ml of fresh media each day for 2 days, and the cultures were sampled again.

TABLE 3. Donor ability of *Proteus lac*⁺ strains*

Donor	Recipient	Selection	Frequency of transfer per donor cell	Remarks
PM-1 F _o - <i>lac</i> ⁺	<i>Escherichia coli</i> K-12 <i>lac</i> ⁻ F ⁻	<i>lac</i> ⁺	1 × 10 ⁻⁴ -3 × 10 ⁻⁶	13-85% of recipients also receive P ⁺ 0-60% of recipients also receive ad ⁺
PM-1 F- <i>lac</i> ⁺	<i>E. coli</i> K-12 <i>lac</i> ⁺ F ⁺	<i>lac</i> ⁺	4 × 10 ⁻⁶ -5 × 10 ⁻⁷	
PM-1 F'-13	<i>E. coli</i> K-12 <i>lac</i> ⁻ P-F ⁻	<i>lac</i> ⁺	2 × 10 ⁻⁷ -1 × 10 ⁻⁸	
PM-1 F'-13	<i>Salmonella typhimurium</i> <i>lac</i> ⁻ ad-F ⁻	<i>lac</i> ⁺	2 × 10 ⁻⁸ -<10 ⁻⁹	

* Ten clones of each of the specified *lac*⁺ *Proteus* strains were mated with recipient cultures as described in Materials and Methods. Hybrid clones were scored for alkaline phosphatase activity (P⁺) by replicating onto plates of low-phosphate minimal medium and spraying the resulting colonies with the substrate *p*-nitrophenyl phosphate. The acquisition of ad⁺ was determined by replication on minimal glucose medium.

TABLE 4. Effect of acridine orange on *Proteus lac*⁺ strains*

Strain	Concn of acridine orange (μg/ml)	No. of colonies examined	No. <i>lac</i> ⁻	Per cent <i>lac</i> ⁻
PM-1 F- <i>lac</i> ⁺	0	659	3	0.41
	20	630	67	11.00
	50	468	122	26.00
PM-1 F'-13	0	1,185	8	0.70
	20	1,145	128	11.00

* Overnight broth cultures of the specified strains were diluted 2 × 10⁻⁵, and 0.1 ml (about 8 × 10³ cells) was inoculated into tubes containing 1 ml of Penassay Broth (pH 7.6) with varying concentrations of acridine orange. The tubes were incubated for 16 hr at 37 C and plated on Mac-Conkey agar.

genes in addition to the *lac*⁺ determinants. All of the *E. coli lac*⁺ hybrids purified from matings with the *Proteus* donors display the properties of strains harboring F-linked genetic determinants. They are heterogenotes, transfer F (or F_o) and *lac*⁺ simultaneously at a high frequency and continually segregate out many types with respect to both fertility and lactose utilization. We conclude that the *Proteus lac*⁺ strains themselves carry genetic elements which exhibit the characteristics attributable to F-linked genes. *Proteus lac*⁺ strains are also infective to other *Proteus* species but not at any higher frequency than to *E. coli*.

One might question that all *Proteus lac*⁺ cells actually carry a linked sex factor, since only a small minority of cells are donors (Table 3). More-

over, *Proteus lac*⁺ populations do not react in antiserum (Orskov and Orskov, 1960) which agglutinates *E. coli*, *Salmonella*, and *Shigella* F⁺ cells, nor are they attacked by a bacteriophage (Loeb, 1960) whose adsorption is dependent upon the presence of the sex factor. On the other hand, *lac*⁺ transfer by *Proteus* can often be increased 50- to 100-fold by simply performing matings directly on agar surfaces rather than in liquid medium. In addition, donor ability and the *lac*⁺ property may be eliminated simultaneously in about 25% of the cells grown from a small inoculum (approximately 10⁴ cells) overnight in 1 ml of Penassay Broth (pH 7.6) containing 20 to 50 μg/ml of acridine orange (Table 4), the conditions specified by Hirota (1960) for the disinfection of F from *E. coli* K-12. It seems likely, therefore, that F is linked to the *lac*⁺ genes in *Proteus* but that the phenotypic surface alterations (Sneath and Lederberg, 1961) associated with F (and presumably necessary for conjugation) are generally reduced or repressed in *Proteus*.

Characterization of DNA from Proteus lac⁺ strains. The DNA extracted from each of the *Escherichia* and *Salmonella* donor strains bands in CsCl with a buoyant density of 1.710 ± 0.001 g/cm³ (equivalent to 50 to 51% GC) with an essentially gaussian, unimodal molecular distribution (Fig. 2A). DNA extracted from *Proteus* species prior to episomal infection exhibits buoyant densities of 1.698 g/cm³ (39% GC) and also shows a small base compositional heterogeneity and a unimodal molecular distribution (Fig. 2B). DNA extracted from *lac*⁺ *Proteus* cells, however, displays a marked molecular heterogeneity (Fig. 2C, D, E). These cells possess, in addi-

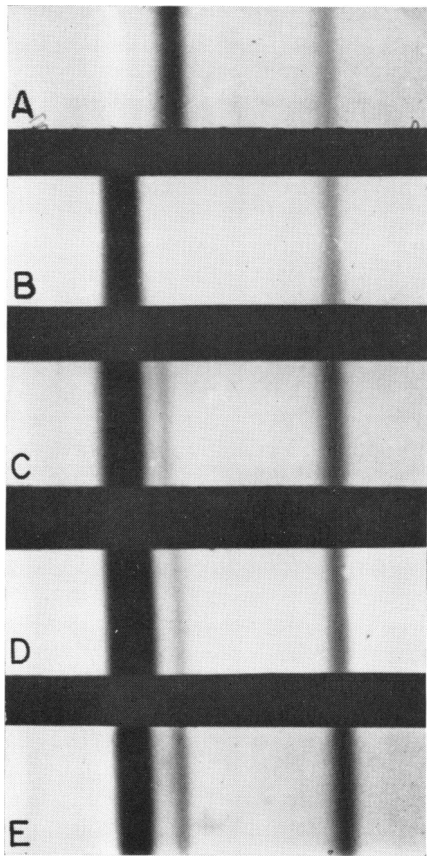


FIG. 2. Molecular heterogeneity of *Proteus lac*⁺ strains. Ultraviolet-absorption photographs taken after centrifugation to equilibrium in CsCl at 44,770 rev/min. The band to the far right in all photographs is the DNA of known density used as a reference. A: *Escherichia coli* K-12 F-*lac*⁺ DNA. B: PM-1 DNA. C: PM-1 F_o-*lac*⁺ DNA. D: PM-1 F-*lac*⁺ DNA. E: PM-1 F'-13 DNA.

tion to the main 1.698 g/cm³ band, an extra or satellite band of DNA with a buoyant density of 1.710 g/cm³. Hence, the buoyant density of the satellite band is equivalent to that of the *E. coli* and *Salmonella* donor strains from which the F-linked *lac*⁺ genes were derived. Figure 3 permits a more quantitative representation of the effects of episomal infection and shows microdensitometric tracings of DNA extracted from the PM-1 strain before and after infection with the F'-13 element. It is clear that the DNA of episodally infected *Proteus* gives rise to two distinct bands in the CsCl density gradient, whereas the uninfected parental *Proteus* strain does not. This result has been repeated on numerous occasions

with each of the representative *lac*⁺ *Proteus* cultures over a period of 12 months with DNA derived from clones which have been through as many as 200 single-colony reisolations.

Various amounts of DNA from *lac*⁺ *Proteus*, ranging from 2 to 6 μg, were centrifuged, and the amount comprising the satellite band was estimated from integration of microdensitometric tracings. Within the limitations of this technique, it was found that the satellite band represented 3 to 4% of the total DNA extracted from *Proteus* F-*lac*⁺ and F_o-*lac*⁺ preparations, while the satellite band present in *Proteus* F'-13 (carrying F-*lac*⁺-P⁺-ad⁺ by genetic tests) extracts comprised 8 to 10% of the total DNA. The relative proportion and density of satellite material is quite stable and characteristic for each type of *lac*⁺ strain.

Associated segregation of lac⁺ and satellite DNA.

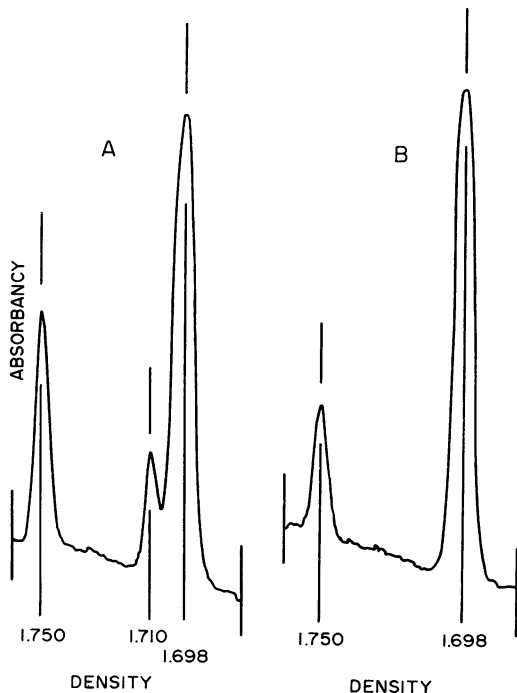


FIG. 3. Effects of episomal infection on PM-1 DNA. Microdensitometer tracings of ultraviolet-absorption photographs taken after equilibrium was reached at 44,770 rev/min. The band of buoyant density 1.750 g/cm³ (deuterated *Escherichia coli* K-12 DNA) is the density standard. A: DNA extracted from PM-1 strain after infection with F'-13 element. B: DNA extracted from PM-1 strain before infection.

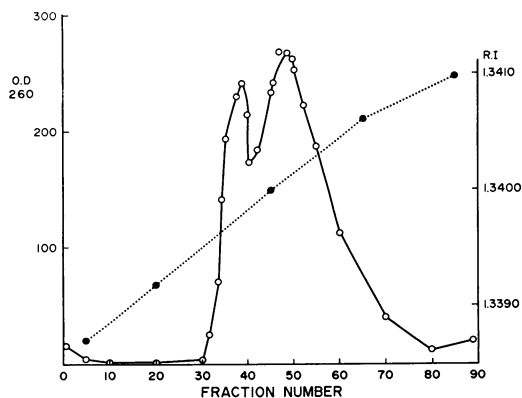


FIG. 4. MAK column chromatography of PM-1 F'-13 DNA. Approximately 1.5 mg of PM-1 F'-13 DNA was diluted in 0.5 M NaCl (buffered to pH 6.8 with 0.05 M phosphate) to 20 μ g/ml and loaded on a MAK column. The DNA was eluted with a NaCl gradient of 0.5 to 0.8 M NaCl and 5-ml fractions were collected. R.I. refers to refractive index measurements used to monitor the gradient (dotted line).

As pointed out previously, despite the daily re-streaking of *Proteus lac*⁺ colonies, they continually segregate out cells of the *lac*⁻ phenotype and may be considered heterogenotes. In *Escherichia* and *Salmonella* strains infected the F-*lac*⁺, F_o-*lac*⁺, and F'-13 elements, a relatively large proportion of *lac*⁻ segregants are found to be *lac*⁻ homogenotes or heterogenotes which have resulted from interaction between the episomic element and the resident chromosome. These F-linked *lac*⁻ markers may be detected by their ability to complement suitable *lac*⁻ mutants (Jacob and Wollman, 1961) or by their reversion to *lac*⁺ on indicator medium (Falkow and Baron, 1962). We applied these methods to the examination of over 100 *lac*⁻ *Proteus* segregants, but none were observed to revert to *lac*⁺ on MacConkey agar or to complement any one of a series of *E. coli* K-12 *lac*⁻ mutants. Similarly, none of the *lac*⁻ *Proteus* cultures could confer fertility to the special indicator strain φ_3 (Richter, 1961), which is an especially sensitive detector of F transmission. Thus, all of the *lac*⁻ *Proteus* segregants examined to date appeared to have completely lost their F-linked genetic elements. Moreover, these segregants accepted F-linked genes in subsequent matings about as readily as did the original *Proteus* recipient strain from which they were descended.

DNA extracted from *lac*⁻ segregants of F-*lac*⁺,

F_o-*lac*⁺, and F'-13 *Proteus* exhibited only one band, of buoyant density 1.698 g/cm³, in CsCl. They were, therefore, indistinguishable from *Proteus* prior to episomal infection. Upon reinfection with an F-linked *lac*⁺ marker, however, their DNA again showed the molecular heterogeneity described previously. Furthermore, if a *lac*⁻ segregant from *Proteus* F'-13 was then infected with a different element (for example, F-*lac*⁺), the satellite band was present in the proportion characteristic of F-*lac*⁺ *Proteus* cells (4%) rather than that of F'-13 *Proteus* cells (8%). If a "second generation" *lac*⁻ segregant was then isolated, once again it was found that the satellite band of DNA had disappeared.

We feel these observations provide strong evidence that the satellite band of DNA associated with episomal infection represents the genetic material of the F-linked *lac*⁺ genes.

Isolation of satellite DNA. It seemed advisable to attempt to fractionate the DNA from *Proteus* carrying F-linked genes to make a closer study of the transferred DNA as well as to provide a more sensitive method for the detection of minor DNA molecular species which might have gone undetected in absorption photographs. The observations of Sueoka and Cheng (1962) that MAK columns can fractionate DNA according to molecular weight, extent of hydrogen bonding, and base composition encouraged us to use this technique. Fig. 4 shows a typical elution profile in which 1.5 mg of PM-1 F'-13 DNA were fractionated from a MAK column with a linear gradient of 0.5 to 0.8 M NaCl. Two distinct peaks can be recognized. Samples of about 3 μ g of DNA from various fractions of the column were subjected to density-gradient centrifugation to identify overall base composition and degree of purity. The earliest DNA fractions eluting from the column were of the highest GC content and represented satellite DNA (Fig. 5). Subsequent fractions contained less of the satellite material.

Although an excellent enrichment of the episome-associated DNA was afforded by MAK fractionation, even the earliest fractions were contaminated to some degree with material from the main (*Proteus*) DNA component. However, the satellite DNA may be isolated in practically pure form by refractionation or altering the gradient of elution, or both. Satellite DNA has an average molecular weight of about 10×10^6 as determined from its sedimentation coefficient ($S_{20,w}$; Doty, McGill, and Rice, 1958) and a buoyant density

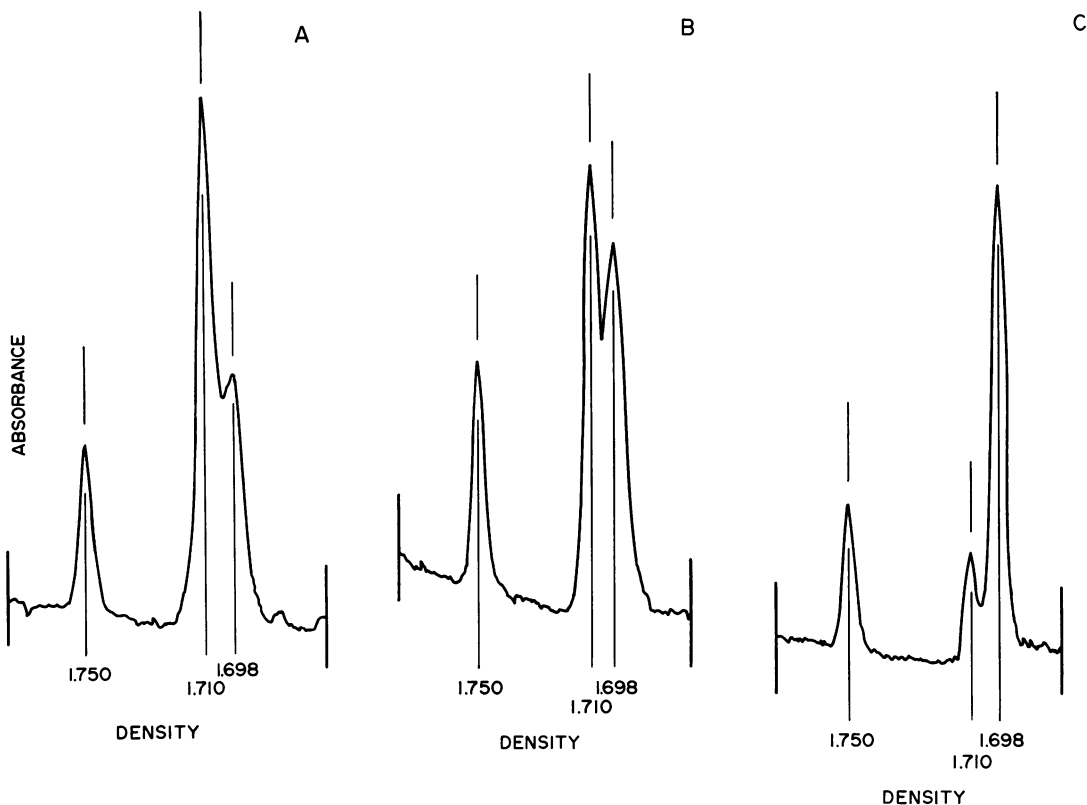


FIG. 5. Fractionation of PM-1 F' -13 DNA. Portions of column fractions shown in Fig. 4 were centrifuged in CsCl at 44,770 rev/min for 20 hr. A: microdensitometric tracing of DNA from fraction 34. B: fraction 38. C: fraction 42. The band of buoyant density 1.750 g/cm³ represents the density standard.

of 1.710 ± 0.002 g/cm³. When the satellite DNA was dissolved in a standard saline-citrate solution (0.15 M NaCl plus 0.015 M Na citrate, pH 7.0), heated to 100 C for 10 min, and quickly cooled in ice water, the buoyant density was found to increase by 0.015 g/cm³. Heated and fast-cooled satellite DNA also showed an increase of 30 to 40% in relative absorbance at 260 μ . Satellite DNA, therefore, exhibits the properties of a highly polymerized, double-stranded DNA molecule (Marmur, Schildkraut, and Doty, 1962).

Essentially identical results were obtained with *Proteus* F-*lac*⁺ and F_o-*lac*⁺ DNA preparations. Also, it should be noted that in no instance could we identify a DNA species with a buoyant density intermediate between those of the main and satellite DNA. If any recombination or stable interaction between the resident chromosome and F-linked *lac*⁺ genes had taken place, an intermediate or "hybrid" DNA band might be expected.

Fractionation of the DNA of the parental *Proteus* strains and several *lac*⁻ segregants resulted in only a single elution peak. Examination of the first DNA fraction eluting from these columns failed to disclose any trace of a molecular species of *Escherichia* base composition.

β -Galactosidase synthesis. The *lac* locus of *E. coli* K-12, as described by Jacob and Monod (1961), contains four genes: *i*, *o*, *y*, and *z*. The genes *z* and *y* provide the genetic information for the structure of β -galactosidase and galactoside permease, respectively. Normally, the *i* gene produces a repressor substance which, by its action on the *o* gene, controls the functioning of the *z* and *y* genes. The presence of lactose or the synthetic sugar IPTG antagonizes the repressor substance and induces enzyme synthesis. Enzyme synthesis in the absence of inducer occurs in strains unable to make repressor (*i*⁻) or in certain *o* mutants (*o*^c), which have become insensitive to repressor.

TABLE 5. β -Galactosidase synthesis by lac^+ *Escherichia coli* and *Proteus* strains*

Strain	Genotype	Enzyme units	
		-IPTG	+IPTG
<i>E. coli</i> K-12 2586 (haploid)	$i^+o^+z^+y^+$	<1	100
<i>E. coli</i> K-12 2586 F- lac^+	$i^+o^+z^+y^-/F-i^+o^+z^+y^+$	<1	309
<i>E. coli</i> K-12 2586 F _o - lac^+	$i^+o^+z^+y^-/F_o-i^+o^+z^+y^+$	23	267
PM-1	?	<1	<1
PM-1 F- lac^+	?/F- $i^+o^+z^+y^+$	16	22
PM-1 F _o - lac^+	?/F _o - $i^+o^+z^+y^+$	36	41

* Strains were grown overnight in synthetic medium plus 0.3% glycerol, with and without 10^{-3} M IPTG. The enzyme level of the haploid strain was taken as a standard for comparison.

TABLE 6. Comparative properties of β -galactosidase isolated from *Escherichia coli* F- lac^+ and *Proteus* F- lac^+ cells

Source	K_M	K_{59C}	SW ₂₀	Immunological unit*
	M	min ⁻¹		
<i>E. coli</i>	1.64×10^{-4}	0.05	16.1	2,781
<i>Proteus</i>	1.71×10^{-4}	0.05	15.4	2,810

* Immunological unit defined as units of enzyme activity precipitated per mg of antibody N at equivalence.

β -Galactosidase synthesis by *Proteus* harboring the various episomic elements was examined to determine whether enzyme synthesis was affected by a diverse cellular environment and whether *Proteus* could correctly interpret the information contained in DNA of different base composition. Table 5 permits a comparison of β -galactosidase production by episomally infected *Escherichia* and *Proteus* strains grown in synthetic medium plus 0.3% glycerol, with and without the inducer IPTG. A typical, inducible *E. coli* haploid strain was also included as a standard. *E. coli* F- lac^+ cells are inducible and produce roughly three times as much enzyme as the haploid strain. This increased synthesis has been interpreted by Jacob and Monod (1961) as indicating the presence of more than one episomic element per chromosome. The F_o- lac^+ element carries an o^c mutation (Falkow and Baron, 1962), and *E. coli* carrying this factor exhibit some constitutive synthesis and a level comparable with F- lac^+ in the presence of inducer.

Proteus strains were normally lac^- and do not produce any detectable β -galactosidase with or without IPTG in the growth medium. *Proteus*

F- lac^+ cells showed constitutive enzyme synthesis and were relatively insensitive to inducer. Moreover, the amount of enzyme produced was only about one-fourth of that produced by the *E. coli* haploid strain. *Proteus* F_o- lac^+ cells showed a somewhat higher level of constitutive enzyme synthesis but were also insensitive to IPTG. These results do not appear to result from the absence of the *i* or *o* genes nor from a permanent modification of any of the F-linked lac^+ genes, since the F- lac^+ and F_o- lac^+ elements transmitted by *Proteus* behaved in the expected manner in *E. coli*. Hence, it appears that the regulatory functions of *Escherichia lac^+ genes may be impaired in *Proteus*. In contrast, as shown in Table 6, the enzyme produced by *Proteus* F- lac^+ cells is essentially identical to that of *E. coli* F- lac^+ cultures in regard to sedimentation constant ($S_{20,w}$), relative affinity of the enzyme for the substrate (K_M), thermal stability at 59 C (K_{59C}), and immunological specificity. Thus, the genetic information for determining the correct structure of *E. coli* β -galactosidase resides in and is apparently correctly interpreted by *Proteus*. A detailed description of enzyme synthesis by episomally infected *Proteus* strains will be reported elsewhere (Wohlhieter et al., *in preparation*).*

DISCUSSION

The results of this investigation show that *Escherichia* genes with the segregation and transfer properties of F-linked markers may be transmitted to species of *Proteus*. *Proteus* strains harboring F-linked lac^+ genes are heterogenotes, limited donors for lac^+ , and may be "cured" of F and the linked chromosomal determinants with acridine orange. *Escherichia* and *Proteus* have very different overall DNA base compositions, and the acquisition of F-linked genes by *Proteus*

may be correlated with the addition of a physically recognizable, high molecular weight DNA fraction with the base composition of *Escherichia*. These results are consistent with the view that the genetic material transferred from *Escherichia* to *Proteus* does not exchange with the resident chromosome but persists in the unintegrated state.

The translating mechanism and enzyme-forming machinery of *Proteus* can correctly interpret the transferred *E. coli* genes and produce β -galactosidase. By all available criteria, this enzyme is found to be indistinguishable from the enzyme formed by *E. coli* K-12. These results imply that the code, by which base sequences in DNA are translated into amino acid sequences in the galactosidase protein, is identical in *Proteus* and *E. coli* regardless of the difference in their overall base compositions. Despite this specificity, the regulatory functions of *Escherichia lac*⁺ genes appear to be impaired in *Proteus*. There is also an apparent impairment of the functioning of the sex-factor in this species. Episomally infected *Proteus* cells are poor donors, and usually do not exhibit the changes in surface properties normally associated with the presence of F. The failure of F to perform efficiently in a species other than *E. coli* K-12 has been observed previously (Makela, Lederberg, and Lederberg, 1962) and, furthermore, the presence of F need not always be manifested by observable fertility in a heterologous species (Luria and Burrous, 1957; Makela et al., 1962). Although we observe these impairments in function, we think it significant that, after an initial instability, the various episomic elements are as stable or more stable in *Proteus* than in many strains of *E. coli* K-12 and more stable than has been observed in most species of *Salmonella* and *Serratia* (see Makela et al., 1962). It is conceivable that, to achieve this stability against a diverse genetic background, the various functions of both the *E. coli* chromosomal genes and episome might be subordinated or altered to the primary requirement of persistence and replication. The "normal" behavior of episomic elements transferred from *Proteus* back to *E. coli* suggests that any changes in the element are not permanent. On the other hand, the frequency of transfer of the episomic elements from *Proteus* back to *E. coli* is so low that we cannot exclude the possibility that those elements transferred by *Proteus* may

represent exceptional back-mutations from a mutant episomal population.

The DNA fractions which may be recognized after the acquisition of *Escherichia* genes by *Proteus* increase with the number of genetic linkage units transferred during conjugation, as evidenced by the size of the satellite band present in DNA from F-*lac*⁺ and F'-13 (F-*lac*⁺-*p*⁺-*ad*⁺) infected cells. It would seem premature, however, to do more than speculate on any relationship between the DNA fraction and the relative contribution of genetic information carried by these molecules. The exact size of a chromosomal segment which may be incorporated by F is not known, nor is there any evidence that the incorporation would be restricted to only completely functional genetic segments. In addition, it is not known whether *Proteus*, as seems to be the case in *E. coli* (Jacob and Wollman, 1961), carries more than one copy of an F-linked marker per chromosome. It would be desirable, of course, to obtain *Proteus* cells infected with F alone. However, unlike F-linked genes which permit the selection of a small number of infected cells, F alone confers no selective advantage and all our attempts, thus far, to obtain *Proteus* F⁺ strains have been unsuccessful. On the other hand, we have recently discovered that it is possible to isolate *Proteus lac*⁺ clones which do not possess even limited donor function. These clones have no discernible satellite band in unfractionated DNA extracts but, upon fractionation on MAK columns, a small satellite band (about 0.3% of the total DNA) of *Escherichia* base composition can still be detected. Although it is not known if any portion of the remaining DNA fraction includes episomal genes, the implication is that the bulk of the DNA we normally associate with F-linked markers represents the fertility component of the element.

Previous studies with episomally infected *Serratia* (Marmur et al., 1961; Falkow et al., 1961) and the results of this investigation demonstrate that intergeneric transfer of genetic material between organisms which differ in DNA base composition represents a unique method of biological fractionation. Jacob and Wollman (1961) pointed out that it is likely that, given a suitable type of Hfr strain, any genetic locus of the bacterial chromosome can be incorporated by the sex factor and be subsequently transferred by conjugation. Transfer of such elements

to *Proteus* (or any other suitable species which possesses a significantly different DNA composition from the donor cell) followed by DNA extraction and fractionation might be used to obtain relatively homogenous species of DNA molecules rich in certain genes or regions of the genome. Such fractions should prove of considerable value for the study of the organization and function of genetic material.

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