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Segregation of Functional Sex Factor into Minicells

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Abstract. The segregation of a bacterial plasmid, the sex factor F′, has been investigated in a cell-division mutant of *Escherichia coli* which produces small anucleate cells (minicells). Significant amounts of isotopically labeled DNA segregate into minicells dependent upon the presence of F′. Minicells containing F′Gal or F′(λ) are shown to donate the plasmid in conjugation. These results demonstrate that the sex factor may be dissociated from the bacterial chromosome and that this separation does not prevent its subsequent transfer.

The precise and equal segregation of genetic elements during cell division assures that the daughter cells are genetically identical. In eukaryotes, this process is accomplished by means of the mitotic apparatus.

The bacterium, *Escherichia coli*, may contain a variety of small, extra-chromosomal genetic elements (i.e., plasmids[1]), some of which may be stably inherited (e.g., the sex factor F, drug resistance transfer factors, colicinogenic determinants, and the prophage P1). Each plasmid constitutes an independent unit of replication or replicon,[2] yet a plasmid/chromosome ratio close to one is generally maintained, and loss of the plasmid is rare. To account for the preservation of this stable relationship, Jacob, Brenner, and Cuzin[2] have suggested that a cell membrane element may connect chromosome and plasmid to form a single unit of segregation. Their suggestion is supported by the direct observation of nuclear material associated with a membranous element, the mesosome,[3] and by experiments showing that a nonreplicating sex factor cosegregates with a previously labeled strand of bacterial chromosome.[4–6]

We have been examining the segregation of plasmids, taking advantage of the special properties of a cell-division mutant of *E. coli* isolated by Adler *et al.*[7] Under a variety of growth conditions, this strain regularly produces small, nondividing, DNA-less cells (minicells) by forming an extra, abnormally located (polar) septum. We have asked whether various plasmids, introduced into the minicell-producing strain, are free to segregate into minicells.[8] Close association of plasmid with chromosome might prevent any such segregation.

In this report, we present evidence that the sex factor, F′Gal, segregates into minicells, and that minicells containing F′Gal or F′(λ) can donate the plasmid to suitable recipients in conjugation. These findings indicate that a functional sex factor may segregate independently of the chromosome.

Materials. Chemicals: Radioisotopes were purchased from New England Nuclear Corp. DNase I was the product of Worthington.

TABLE 1. *List of strains.*

Strain	Other pertinent markers	Use
MIN (=P678-54 λ^S)	F$^-$-Gal$^-$-Thr$^-$-Leu$^-$-Thi$^-$-T6^S	Labeling experiment
MIN/F'$_8$Gal		Labeling experiment
MIN (λcI*857*)λ^R/F'$_8$Gal		Donor of F'Gal
MIN (λcI*857*)/F'Gal(λcI*857*)		Donor of F'(λ)
W3350 λ^RT6^R	F$^-$*gal* K$^-$T$^-$	Recipient of F'Gal
W3102	F$^-\lambda^S$	Recipient of F'(λ)
AB1157 (*λind*$^-$)	F$^-$Gal$^-$Pro$^-$Thr$^-$Leu$^-$His$^-$Arg$^-$Thi$^-$	Recipient of F'Gal(λ)
Hfr Hayes		Plating strain

Sex factors: F'$_8$Gal (Hirota and Sneath[13]) does not contain the λ prophage attachment site; the other F'Gal (isolated by K. Matsubara; see ref. 6) does. Prophages: λcI*857* is a thermoinducible and *λind*$^-$ an uninducible mutant. Abbreviations: Gal$^-$, galactose nonfermenting; Pro$^-$ Thr$^-$Leu$^-$His$^-$Arg$^-$Thi$^-$, requirements for proline, threonine, leucine, histidine, arginine, and thiamine, respectively; *gal* K$^-$T$^-$ is a kinase-less, transferase-less double mutant; λ^R, λ^S, T6^R, T6^S, resistant and sensitive to phages λ and T6.

Liquid media: Minimal: 0.7% Na_2HPO_4, 0.3% KH_2PO_4, 0.1% NH_4Cl, 10^{-3} M $MgSO_4$, 0.4% glycerol, 0.25% casamino acids, and 1.0 μg/ml thiamine hydrochloride. D medium:[9] 0.3% nutrient broth (Difco), 0.5% tryptone (Difco), 0.5% NaCl, and 1.0 μg/ml thiamine hydrochloride. Tryptone-maltose: 1% tryptone, 0.5% NaCl, 0.2% maltose, 10^{-2} M $MgSO_4$, and 1.0 μg/ml thiamine hydrochloride.

Plating media: Tryptone agar: 1% tryptone, 0.25% NaCl, 1.0 μg/ml thiamine hydrochloride, and 1.1% agar. Tryptone overlay agar: 1% tryptone, 0.25% NaCl, and 0.7% agar. Galactose indicator agar: MacConkey agar of the Difco Manual (Difco Laboratory, Detroit, Mich.) with galactose substituted for lactose. EMBO agar is the EMB agar of Campbell,[10] with sugar omitted. Minimal media for selective platings contained medium E[11] or M56[12] used half strength, 2% agar, and appropriate supplements. Minimal overlay agar: 0.6% agar.

Bacterial strains: All strains used are derivatives of *E. coli* K12 (Table 1). A λ-sensitive derivative of the minicell-producing strain of Dr. Howard Adler, P678-54,[7] was obtained from Dr. J. L. Rosner. This strain, hereafter designated MIN, was the source of all other minicell-producing strains used here. Standard procedures for bacterial mating and lysogenization were used in constructing strains.

Methods. Determination of bacterial characteristics: The following markers were tested for in the following manner: (*a*) *gal*$^+$, by plating on minimal galactose agar or on galactose indicator agar; (*b*) maleness, by sensitivity to the male-specific phage, f2;[14] (*c*) prophage λcI*857*, by thermal inducibility; (*d*) F'Gal, by linked transfer of *gal*$^+$ and maleness to a *gal*$^-$ female; (*e*) F'(λ), by zygotic induction of λ on mating with W3102.

Purification of minicells: This procedure is a modification of those described by Adler *et al.*[7,15] (*a*) Differential centrifugation: Centrifugation of cultures at 1600 $\times$ *g* for 8 min sediments approximately 90–95% of the viable bacteria. Minicells are then obtained from the supernatant by centrifugation at 10,000 $\times$ *g* for 10 min. Where lower viable cell contamination was desired, this cycle of low and high speed centrifugations was repeated prior to sucrose gradient sedimentation. (*b*) Sucrose gradients: The sample (1.2–1.8 ml) containing partially purified minicells was layered atop a 31.5-ml linear gradient of 5–20% sucrose in 0.067 M sodium phosphate, pH 6.8, in a polyallomer tube (Beckman). The gradient was centrifuged for 50 min at 2200 rpm (1000 $\times$ *g*) in the 269 rotor of the International PR-II centrifuge. Under these conditions, most of the remaining bacteria sediment to the bottom of the tube. The minicells, clearly identifiable as a discrete band in the upper third of the tube, are collected by lateral needle puncture of the gradient just below the minicell band. After centrifugation (10,000 $\times$ *g* for 10 min), the minicells are resuspended in 0.8–1.0 ml of growth medium and resedimented in a second identical sucrose gradient. Individual fractions are collected by gravity drainage through a needle laterally introduced into the tube just above the bottom.

All centrifugations are conducted at 0–3°C.

Assays of bacteria, minicells, and infective centers: Viable bacteria were assayed as colony-forming units on tryptone agar. Plates were counted after 24 hr. Longer incubation or use of enriched media (nutrient agar (Difco) or LB-agar[16]) did not affect the results. Minicells were measured turbidimetrically at 650 nm. By a calculation based upon the relative volumes of minicells and viable cells,[7] one may roughly estimate that an optical density of 0.3 represents approximately 10^9 minicells. Infective center assays were performed according to the method of Adams.[17]

Counting of labeled cells and minicells: Samples were prepared for counting by boiling for 2 min followed by the addition of an equal volume of ice-cold 10% trichloroacetic acid. Samples were collected on Whatman GF/C glass fiber filter discs, washed with 5 ml cold 10% trichloroacetic acid and then with 10 ml ethanol (99%). After drying under a heat lamp, the filters were counted in a toluene-base scintillator (Liquifluor; Pilot Chemicals, Inc.). At settings for double-label counting, the efficiency for ^3H is 9%, for ^{14}C, 65%.

Tests for phage sensitivity: Colonies to be tested were transferred with a sterile toothpick to an EMBO plate spread with T6 (4×10^9 plaque-forming units) or with f2 (3×10^{10} plaque-forming units). After overnight incubation at 40°C, phage-sensitive colonies were very small and dark; phage resistant, large and pink.

Centrifugation of DNA: DNA was isolated from minicells and sedimented in alkaline sucrose gradients by the methods of Freifelder.[18] Equilibrium centrifugation in CsCl-ethidium bromide was performed according to the techniques of Cozzarelli *et al.*[19]

Results. Preferential segregation of labeled DNA into minicells from an F′-carrying strain: Evidence for the segregation of sex factor into minicells was first sought in experiments in which cellular DNA was isotopically labeled. Isogenic male (F'_8Gal) and female (F^-) minicell-producing strains were labeled by growth for four generations in ^3H- and ^{14}C-thymidine, respectively. The cells were washed free of unincorporated label, diluted into fresh media, and allowed to grow and to produce minicells over several generations. The cultures were then combined, and the minicells purified by differential centrifugation and by sedimentation in sucrose gradients. The gradient fractions were examined for the presence of acid-precipitable radioactivity.

The result of a typical experiment is shown in Figure 1. A small fraction of the incorporated ^{14}C, not attributable to contaminating viable bacteria, sedimented with the minicells (absorbance), suggesting that even in the female strain, some DNA can be trapped in minicells. A much larger fraction of incorporated radioactivity (^3H) sediments with the minicells produced by the F′-carrying strain. The proportions of ^3H:^{14}C:viable bacteria (each expressed as percentage of the total) in the peak for residual viable bacteria (fraction 8) are close to the expected values of 1:1:1. In contrast, the proportions become 60:9:1 in the peak for minicells (fraction 14). The radioactive label (both ^3H and ^{14}C) resides in intracellular DNA: it was rendered acid-soluble by DNase, but only after the minicells had been lysed. The preferential enrichment of ^3H-labeled DNA in the minicell fractions is presumptive evidence for the segregation of F′Gal into minicells.

Rough estimates may be made of the amount of F′DNA segregating into minicells. Of the total incorporated radioactivity, an average (seven experiments) of 0.08% was recovered in minicells after seven generations; this value has already been corrected for the small amount recovered in the F^- strain. Thus, approximately 4% of the originally labeled F′DNA (the molecular weight

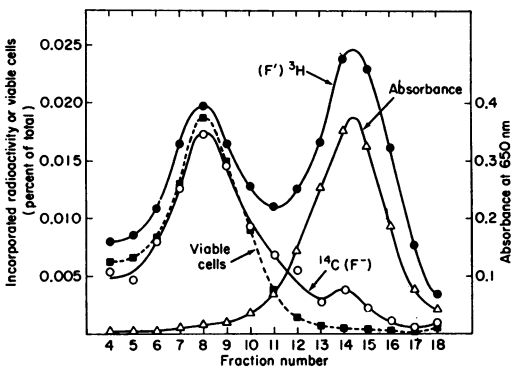

FIG. 1.—Segregation of labeled DNA into minicells from (F′) and (F$^-$) strains. The minicell-producing strain (F$^-$) and its F′$_8$Gal$^+$ homologue were grown in minimal medium at 37°C to a titer of 8×10^8/ml. The DNA of each culture (5 ml) was labeled by growth in the presence of radioactive thymidine (F$^-$: ^{14}C-thymidine, 2.2 μCi/ml, spec. act. 30 μCi/μmol; F′: ^{3}H-thymidine, 20 μCi/ml, spec. act. 6700 μCi/μmol) and 100 μg/ml deoxyadenosine.[20] After labeling for four generations, the cells were collected by centrifugation, washed, and diluted into fresh media (115 ml) containing 100 μg/ml unlabeled thymidine, 1 mM $MgSO_4$, and 40 μg/ml DNase (Worthington) to degrade adventitious extracellular DNA. The two cultures were allowed to grow separately and to produce minicells for six generations (final titers, 4×10^8 viable cells/ml). The cultures were chilled, combined, and the minicells purified by differential centrifugation and two sucrose gradients (see *Methods*). The analysis of the second sucrose gradient is shown in this figure. Each fraction (18 collected) contained 1.75 ml. The absorbance peak is due to minicells. Counting of radioactivity and plating for viable cells is described under *Methods;* results are expressed as percentage of the total incorporated radioactivity or of total viable cells present at the end of the segregation period. Total incorporated $H^3 = 1.76 \times 10^6$ cpm, $^{14}C = 4.5 \times 10^5$ cpm; total number of viable bacteria, 9.7×10^{10}.

of F′$_8$ Gal is about 2% that of the chromosome[21]) segregates into minicells in seven generations, or 0.5% per generation. Roughly 1% of minicells receive F′Gal (each generation, half the MIN cells give rise to a minicell). These are to be taken as order of magnitude calculations; they ignore losses of minicells during centrifugation, and they assume that all male strain specific DNA in minicells is F′DNA and that the sex factor/chromosome ratio in the F′ strain is one.

When DNA found in minicells formed by the F′ strain was examined by sedimentation in alkaline sucrose gradients and by density gradient centrifugation in cesium chloride containing ethidium bromide, a small fraction (1-3%) of the total minicell radioactivity behaved as covalently circular DNA. The reason for this low yield is unclear.

Conjugal transfer of F′Gal from minicells: Recovery of biologically functional sex factor from minicells would provide the most compelling evidence for its presence. Purified minicells, produced by the F′$_8$Gal$^+$-containing strain, were mated with a *gal*$^-$ female, and the exconjugants were examined for conversion of females to *gal*$^+$. The purification of donor minicells (Table 2) was facilitated by lysogenizing the minicell-producing strain with a heat-inducible prophage (λc*I857*).[22] The number of contaminating viable bacteria was then greatly reduced by thermal induction. As seen in Table 2, the number of females converted to *gal*$^+$ was 500-fold greater than could be attributed to transfer of *gal*$^+$ from the few residual viable bacteria. The *gal*$^+$ colonies were all T6-resistant, like the original female; they were all (108/108) sensitive to the male-specific phage, f2, indicating that they had received the sex factor F′ in addition to the *gal*$^+$ marker. Secondary transfer of F′Gal was demonstrated with these colonies as donors.

TABLE 2. *Purification of minicells capable of donating F'Gal.*

	Total viable bacteria	Donors of Gal$^+$
Original culture	1.8×10^{-10}	2.0×10^{10}
After heat induction	1.4×10^{6}	
After differential centrifugation (2 cycles)	5×10^{3}	
Minicell fraction from sucrose gradient	<8	4000

The male minicell-producing strain, MIN ($\lambda cI857$)/F'$_8$Gal, was grown to a titer of 8×10^7/ml in D medium at 34°C. The culture was then placed at 41°C for 70 min to kill the majority of normal-sized cells by thermal induction of the prophage λ. Following two cycles of differential centrifugation (*Methods*), the minicells were further purified by sedimentation in a sucrose gradient. The barely visible minicell band was collected as one fraction, centrifuged free of sucrose, and resuspended in D medium. After plating an aliquot to determine the titer of viable donor bacteria, 8×10^7 female *gal*$^-$ bacteria (W3350 λ^R T6^R) were added, and the mating mixture incubated at 37°C without shaking for 70 min. A parallel mating with viable cells as donors was performed, using an appropriate dilution of the original culture (line 1). Mating was interrupted by vigorous agitation. The number of recipients converted to *gal*$^+$ was determined by plating on minimal-galactose plates at 40°C; the *thr*$^-$ *leu*$^-$ minicell-producing parent is selected against on this medium. The plating efficiency of each parent on minimal-galactose medium is $<5 \times 10^{-9}$.

Further evidence that minicells contain a functional sex factor was obtained by a sedimentation analysis of the distribution of "donor activity" (Fig. 2). The ability to donate F'Gal in mating was found to copurify with the minicells. The *gal*$^+$ colonies were again f2-sensitive and capable of secondary transfer of F'Gal. This result shows that transfer was not due to nonviable (i.e., not colony-forming) but fertile donor bacteria of normal size; the size distribution of any such nonviable but fertile cells would be expected to parallel that of the viable cells.

Table 3 presents evidence against the even more remote possibility that the donor ability resides in a special class of exceptionally small, chromosome-containing yet nonviable bacteria. The male minicell-producing parent, like other F' strains,[23] transfers chromosomal markers at moderate frequency (Table 3, col. 1). In contrast, the minicell donor of F'Gal does not (col. 2).

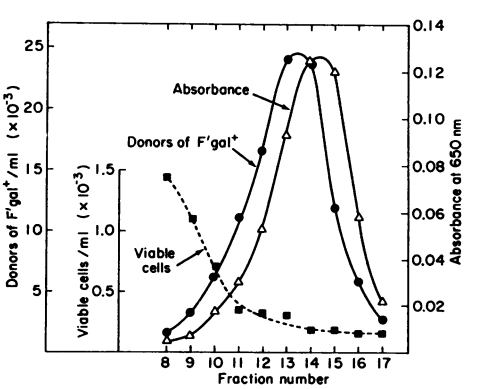

FIG. 2.—Copurification of donor activity (F'Gal) and minicells on a sucrose gradient. MIN ($\lambda cI857$)/F'$_8$Gal$^+$ was grown in D medium (400 ml) at 34° to 2×10^9/ml. Minicells, partially purified by two cycles of differential centrifugation and a heat step (20 min at 41°C followed by 50 min at 37°C), were sedimented in a sucrose gradient and 18 fractions (1.75 ml) collected. Portions of each indicated fraction were centrifuged (10 min at 10,000 x *g*), and each minicell pellet resuspended in D medium (0.3 ml). Aliquots were plated at 34°C for viable bacteria. Female *gal*$^-$ bacteria (W3350 λ^RT6^R) were then added (1×10^8 cells) to each fraction, and the mating mixtures (0.4 ml) were incubated with very gentle shaking at 37°C. After 90 min, the mating mixtures were diluted and vigorously agitated to terminate mating. Recipients converted to *gal*$^+$ were detected by plating on minimal-galactose plates at 40°C.

TABLE 3. *Minicells harboring F'Gal(λcI857) do not transfer chromosomal markers.*

Transferred marker	Heat-Resistant Exconjugants/ml with the Selected Marker	
	Bacterial donor ($\times 10^{-5}$)	Minicell donor ($\times 10^{-3}$)
Episomal *gal*$^+$	160	187
Chromosomal *pro*$^+$	3.5	<0.01

The donor in mating was either MIN (λcI857)/F'Gal(λcI857) at 3.2×10^7 viable cells per ml of mating mixture or purified minicells (OD_{650} 0.10/ml of mating mixture) derived therefrom (see Legend to Fig. 3). The recipient was the *gal*$^-$ *pro*$^-$ female AB1157 (λ*ind*$^-$) (1.7×10^8 viable cells/ml). Mating mixtures were incubated at 33°C for 80 min and plated for recipients converted to *gal*$^+$ or *pro*$^+$ on selective media at 40°C. On plating the unmated parents, no colonies appeared.

The best preparation of minicells effected 7.8×10^5 transfers of F'Gal per absorbance (650 nm) of 0.15, i.e., per 5×10^8 minicells. Since approximately 1% of the minicells carry F'Gal (see above), approximately 15% of the F'-containing minicells gave effective transfers.

The finding of Cohen *et al.*[15] that minicells derived from male strains cannot serve as recipients in mating rules out the possibility that the functional sex factor enters the minicell not by segregation but by mating with viable donors.

Conjugal transfer of F'(λ) from minicells: In subsequent experiments designed to examine whether the prophage λ could be induced in minicells, minicells were obtained from a strain carrying a sex factor lysogenized with the temperature-inducible mutant of λ, λcI857. These minicells did plate as infective centers on the λ-sensitive female strain, W3102 (Fig. 3), but not on the related λ-sensitive male strains (see legend to Fig. 3). Moreover, the minicell infective centers were nearly as numerous at 34°C (no thermal induction) as at 40°C. The infective centers were clearly the result of zygotic induction, following transfer of F'(λ) from minicells to λ-sensitive females, occurring on the plate. Zygotic induction of λ is independent evidence for the presence of biologically functional F' in minicells.

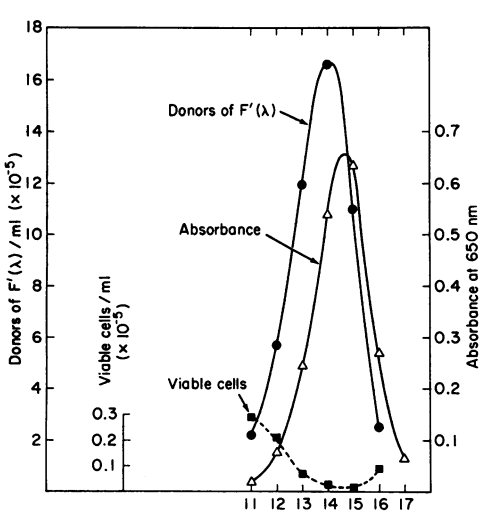

FIG. 3.—Copurification of donor activity (F'[λ]) and minicells on a sucrose gradient. MIN (λcI857)/F'Gal (λcI857) was grown in tryptone-maltose medium at 34°C to a titer of 3×10^8/ml. Minicells were purified by differential centrifugation and two sucrose fractionations, the second of which is shown (18 fractions collected). Aliquots of the indicated fractions, appropriately diluted, were plated for viable bacteria at 34°C and for infective centers on λ-sensitive F$^-$ indicator bacteria (W-3102) at 40°C. When infective centers were assayed on a male strain (Hfr Hayes), titers were lower by more than 100-fold.

The minicells from pooled fractions 13–15 were freed of sucrose by centrifugation, resuspended in tryptone broth containing 10^{-3} $MgSO_4$ and 15% glycerol, and frozen and stored at −70°C, conditions under which donor activity is preserved. A portion of these minicells was used in the experiment described in Table 3.

Discussion. We have presented chemical and biological evidence for the segregation of an extrachromosomal replicon, F′, into minicells. We have also shown that the separation of the sex factor from the chromosome does not prevent its subsequent transfer. These results were unexpected in the light of previous evidence for strict cosegregation of the sex factor and the bacterial chromosome.[4–6] Two explanations for this apparent discrepancy may be considered. (1) F is not free in the cytoplasm but is joined by a common element to the chromosome with which it cosegregates. Occasionally, F and the chromosome are separated by the plane of abnormal cell division; the result is a minicell containing F and a normal-sized cell which has lost F. The F in the minicell would enter and remain bound to its membranous (or other) site. A free cytoplasmic existence for F need not be postulated. (2) F can exist both free in the cytoplasm and bound to the membrane. When truly cytoplasmic, it will on occasion be trapped in a newly forming minicell. It is membrane bound only temporarily unless its replication is blocked.

It will be recalled that the evidence for strict cosegregation of the sex factor and the chromosome was obtained in experiments which employed a nonreplicating F′. Cuzin and Jacob used a mutant sex factor thermosensitive for its own replication, while Hohn and Korn studied the segregation of a sex factor whose replication was completely blocked by acridine orange. It is tempting to speculate that these findings of strict cosegregation are necessarily dependent upon a nonreplicating sex factor, and that only a replication-inhibited sex factor remains permanently bound to its membranous site waiting vainly to be copied. Studies on the segregation into minicells of F′ thermosensitive for replication may test this hypothesis.

The segregation of the sex factor into minicells provides a potential source of sex-factor DNA free of chromosomal contamination. At present, the extent of F′ segregation is too low to be conveniently used for preparative purposes, but isotopically labeled material can be prepared for analytical purposes (e.g., hybridization).

There is considerable controversy in the literature concerning the role of DNA synthesis in bacterial conjugation.[24] The important question of whether DNA synthesis in the donor is necessary to drive conjugal transfer remains unanswered. Because of the negligible background of chromosomal DNA, matings with minicells as donors should provide a suitable system for approaching this question.

The finding of DNA in the minicells of the female strain deserves some comment. Adler *et al.*, using chemical methods, concluded that there was virtually no DNA in minicell preparations that could not be accounted for on the basis of contaminant viable bacteria.[7] The greater sensitivity afforded by isotopic methods has permitted the detection of small amounts of DNA in the minicells of the female strain. This result supports earlier work by Tudor *et al.*[25] who obtained electron microscopic evidence for the presence of nuclear material in minicells. Little has been done to characterize this material. By sedimentation analysis in alkaline sucrose gradients, it appears to be of low molecular weight.

The method used here to study F′ segregation has general applicability. With it we have shown that nonintegrated λ segregates into minicells whereas the

extra-chromosomal prophage P1 does not.[8] Recently, Levy has obtained evidence to suggest that transferable R factors segregate into minicells.[26]

The minicell containing a transferable sex factor is a nonviable vector for genetic recombination. To view it as a primitive bacterial analogue of a spermatozoan, though fanciful, illustrates how mutation might lead to the formation of specialized, nonviable units for promoting genetic exchange. A stronger analogy may exist between the fertile minicell and the temperate bacteriophage, each vectors of genetic exchange which cannot independently replicate. Some have speculated that a bacteriophage may represent a plasmid which selected a protein coat from among various bacterial constituents, and thus grew by accretion. Alternatively, a bacteriophage may have developed from a plasmid-containing anucleate cell by a process involving progressive simplification.

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[1] Lederberg, J., *Physiol. Rev.*, **32**, 403 (1952).
[2] Jacob, F., S. Brenner, and F. Cuzin, *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 329.
[3] Jacob, F., A. Ryter, and F. Cuzin, *Proc. Roy. Soc. B*, **164**, 267 (1966).
[4] Cuzin, F., and F. Jacob, *Compt. Rend.*, **260**, 5411 (1965).
[5] Cuzin, F., and F. Jacob, *Ann. Inst. Pasteur*, **112**, 529 (1967).
[6] Hohn, B., and D. Korn, *J. Mol. Biol.*, **45**, 385 (1969).
[7] Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree, these PROCEEDINGS, **57**, 321 (1967).
[8] Kass, L. R., manuscript in preparation.
[9] Devoret, R., M. Monk, and J. George, *Zentralbl. Bacteriol. Orig.*, **196**, 193 (1965).
[10] Campbell, A., *Virology*, **4**, 366 (1957).
[11] Vogel, H. J., and D. M. Bonner, *J. Biol. Chem.*, **218**, 97 (1956).
[12] Monod, J., G. Cohen-Bazire, and M. Cohn, *Biochim. Biophys. Acta*, **7**, 585 (1951).
[13] Hirota, Y., and P. H. A. Sneath, *Jap. J. Genet.*, **36**, 307 (1961).
[14] Loeb, T., *Science*, **131**, 932 (1960).
[15] Cohen, A., W. D. Fisher, R. Curtiss, III, and H. I. Adler, these PROCEEDINGS, **61**, 61 (1968).
[16] Luria, S. E., J. N. Adams, and R. C. Ting, *Virology*, **12**, 348 (1960).
[17] Adams, M. H., *Bacteriophages* (New York: Interscience Publishers, Inc., 1959).
[18] Freifelder, D., *J. Mol. Biol.*, **34**, 31 (1968).
[19] Cozzarelli, N. R., R. B. Kelly, and A. Kornberg, these PROCEEDINGS, **60**, 992 (1968).
[20] Boyce, R. P., and R. B. Setlow, *Biochim. Biophys. Acta*, **61**, 618 (1962).
[21] Freifelder, D., *J. Mol. Biol.*, **35**, 95 (1968).
[22] Rosner, J. L., E. A. Adelberg, and M. B. Yarmolinsky, *J. Bacteriol.*, **94**, 1623 (1967).
[23] Pittard, J., J. S. Loutit, and E. A. Adelberg, *J. Bacteriol.*, **85**, 1394 (1963).
[24] Curtiss, R., III, *Ann. Rev. Microbiol.*, **23**, 69 (1969).
[25] Tudor, J., T. Hashimoto, and S. F. Conti, *J. Bacteriol.*, **98**, 298 (1969).
[26] Levy, S. B., manuscript in preparation.