TRANSFER-DEFECTIVE MUTANTS OF SEX FACTORS IN *ESCHERICHIA COLI*. II. DELETION MUTANTS OF AN F-PRIME AND DELETION MAPPING OF CISTRONS INVOLVED IN GENETIC TRANSFER

EIICHI OHTSUBO

Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka, 560 Japan

Received May 26, 1969

IN the preceding paper (OHTSUBO, NISHIMURA and HIROTA 1970), at least seven cistrons involved in genetic transfer directed by F and R factors were identified by complementation analysis between transfer-defective mutants of F and R factors. The sequence of these cistrons on the F or R factor, however, was not determined.

An R factor can be transduced by phage P1*kc*, which sometimes produces deletion mutations affecting the drug resistance markers (WATANABE and FUKASAWA 1961) or in the genes controlling conjugal fertility (SUGINO and HIROTA 1962). It has also been found that the sex factor moiety of an F-prime is transduced by phage P1*kc* jointly with exogenote genes associated with it (FRÉDÉRICQ 1965; HIROTA personal communication). These facts suggest the possibility that transfer-defective deletion mutants of an F-prime may be isolated by transduction with phage P1*kc*. Such mutants, if isolated, can be utilized for the mapping of the cistrons involved in genetic transfer. Transfer-defective mutants, in fact, can be readily isolated by transduction to a rec$^-$ recipient in which the integration of transducing fragments into the chromosome is inhibited.

In this paper, isolation and characterization of deletion mutants of F_8, an F-prime carrying galactose genes (HIROTA and SNEATH 1961), and mapping of six cistrons involved in genetic transfer are reported.

MATERIALS AND METHODS

Bacteria, transfer-defective R factors, and phages: The strains of *E. coli* K12 used in these experiments are listed in Tables 1a and 1b. Transfer-defective R factors used were $R_{100-300}$, R_{100-67}, R_{100-73}, R_{100-75}, R_{100-69}, and R_{100-70} which belong to cistrons, *A*, *B*, *C*, *D*, *E*, and *F*, respectively. Their derivations and characters were described in the preceding paper (OHTSUBO, NISHIMURA and HIROTA 1970).

Male-specific phages used were M12, MS2, f2, Qβ, f1, and ZD and the female-specific phage used was *tau*(τ) (OHTSUBO, NISHIMURA and HIROTA 1970). Phage P1*kc* (LENNOX 1955) has been used in transduction experiments.

Detection of F gal *mutants with defects in galactose operon and analysis of* galK *mutations by spot tests:* A culture of bacteria harboring F *gal* was spotted on streaks of known *gal*$^-$ cultures and Gal+ prototrophs were selected on EM-galacotse-minimal agar. Numerous Gal+ papillae are a good indication that complementation has occurred, and a reduced yield of Gal+ papillae sug-

TABLE 1a

E. coli *K12 strains used in transduction experiments*

Strain	Characteristics*	Source or reference
W4520	F_8+† *met*	HIROTA and SNEATH (1961)
JE170	F- R_{100-1}+‡ *lac-11 mal-5 str*	EGAWA and HIROTA (1962)
W4573	F- *galK-2 lac-85 ara-2 xyl-2 str*	LEDERBERG (1960)
W3623	F- *galT-6 trp str*	LEDERBERG (1960)
W3110	F- *gal*+	LEDERBERG (1960)
W3623 *recA*	Same as W3623 except *recA arg*	OGAWA, SHIMADA and TOMIZAWA (1968)
W3623 F_8+	Same as W3623 except F_8+	F_8 transfer from W4520
W3623 *recA* F_8+	Same as W3623 *recA* except F_8+	F_8 transfer from W4520
W3623 R_{100-1}+	Same as W3623 except R_{100-1}+	R_{100-1} transfer from JE170
JE3264	F- *galK-2 galT-1 lac*	a *lac*- mutant of W3350
JE3264 F_8+	Same as JE3264 except F_8+	F_8 transfer from W4520

Genetic symbols are those used by TAYLOR and TROTTER (1967).
* Suffixes of symbols signify independent origin of mutations.
† F_8 is an F-prime carrying a galactose operon.
‡ R_{100-1} is an R factor derepressed for fertility.

gests that complementation has not occurred. F *gal* mutants which did not complement *galK* mutants were detected in such a way. Next, the strains harboring these F *gal* mutants were analyzed for mating with a number of *galK* mutants by spot tests as described above. When the F gal donors were recA- from which the transfer of host *gal* genes was inhibited (CLOWES and MOODY 1966; WILKINS 1969), no or a reduced but considerable yield of Gal+ papillae were formed on the spots. The latter indicates that the F *gal* covers the *galK* mutation and recombi-

TABLE 1b

*Galactose negative strains**

Strain	Mutation	Strain	Mutation
	Kinase (*galK*) mutants		
W3102	*gal-2*	W3748	*gal-12*
W3108	*gal-8*	W3965	*gal-14*
W4670	*gal-10*		
	Transferase (*galT*) mutants		
W3101	*gal-1*	W3104	*gal-4*
W3991	*gal-1*	W3107	*gal-7*
	Epimerase (*galE*) mutants		
W3995	*gal-22*		
	Kinase-transferase double mutant		
W3350	*gal-1 gal-2*		
	Triply defective mutant		
W3109	*gal-9*		

* All mutants were isolated and described by MORSE, LEDERBERG, and LEDERBERG (1956a,b) and LEDERBERG (1960).
Kinase, transferase, and epimerase are abbreviations of galactokinase, galactose-1-phosphate uridyl transferase, and UDP-galactose 4-epimerase, respectively. *galK*, *galT* and *galE* are cistrons specifying these enzymes.

nation has occurred. Thus, with various F *gal* mutants, various *galK* mutations were classified and mapped by the method of overlapping deletions.

Complementation analysis of transfer cistrons: A set of transfer-defective R mutants were transduced by phage P1kc into the cells harboring transfer-defective F_8 (OHTSUBO, NISHIMURA and HIROTA 1970). The culture of bacteria harboring both factors was cross-brushed on streaks of a F⁻ R⁻ Gal⁻ recipient culture (W4573) and Gal⁺ prototrophs were selected on EM-galactose-minimal agar. Numerous Gal⁺ papillae indicates that complementation has occurred between defective F_8 and defective R factors and a few Gal⁺ papillae indicates that complementation has not occurred (OHTSUBO, NISHIMURA and HIROTA 1970).

For *media and methods of the other experiments,* see the preceding paper (OHTSUBO, NISHIMURA and HIROTA 1970).

RESULTS

*Transduction of F_8 by phage P1*kc: When both the donor and recipient had the same galactose-negative mutation in their chromosomes and the donor carried an F_8 *gal*⁺ exogenote, only the exogenote galactose genes on F_8 were scored in Gal⁺ transductants.

With W3623 F⁻ *galT-6 rec*⁺ and W3623 F⁻ *galT-6 recA*⁻ as gal⁻ recipients and W3623 F_8⁺ *gal*⁺/*galT-6 rec*⁺ as a donor, Gal⁺ transductants were formed in both recipients. However, with the rec⁻ recipient one hundred fewer transductants were formed. When W3110 F⁻ *gal*⁺ *rec*⁺ was a donor, in which wild-type *gal* genes were chromosomal, no Gal⁺ transductants appeared in the rec⁻ recipient. A chloramphenicol resistance marker (Cm^R) of a drug resistance factor, R_{100-1} which replicates autonomously, could be transduced into the rec⁻ recipient as efficiently as into the rec⁺ recipient (Table 2). It seems, therefore, that only *gal*⁺ genes capable of replicating autonomously can be scored as Gal⁺ transductants in the rec⁻ recipient.

Transfer ability for the *gal* genes of the transductants was tested. In the rec⁺ recipient about 50 percent of the gal⁺ transductants had transmissible F *gal*, while in the rec⁻ recipient 94 percent contained transmissible F *gal* (Table 3).

TABLE 2

*Transduction of episomal or chromosomal genes into rec⁺ or rec⁻ recipients with phage P1*kc

Donor*		Recipient†		Gene transduced	Transduction frequency‡
W3623	rec⁺ F_8⁺	W3623	rec⁺	Episomal gal⁺	3.7×10^{-6}
W3623	rec⁺ F_8⁺	W3623	rec⁻	Episomal gal⁺	3.3×10^{-8}
JE3264	rec⁺ F_8⁺	JE3264	rec⁺	Episomal gal⁺	1.4×10^{-6}
W3110	rec⁺ F⁻	W3623	rec⁺	Chromosomal gal⁺	5.5×10^{-6}
W3110	rec⁺ F⁻	W3623	rec⁻	Chromosomal gal⁺	$< 10^{-8}$
W3623	rec⁺ R_{100-1}⁺	W3623	rec⁺	Episomal Cm^R	4.0×10^{-5}
W3623	rec⁺ R_{100-1}⁺	W3623	rec⁻	Episomal Cm^R	9.4×10^{-6}

According to the method by LENNOX (1955); gal⁺ or Cm^R transductants formed on DAVIS-minimal sugar-agar (DAVIS and MINGIOLI 1955) containing necessary amino acid, sugar, or antibiotic where selected.

* All host bacteria of donor phage P1*kc* were λ nonlysogenic.

† W3623 has a gal⁻ mutation, *galT-6*. JE3264 has two mutations, *galK-2* and *galT-1*.

‡ Frequencies of transduction were expressed as the number of gal⁺ or Cm^R colonies formed per donor phage P1*kc*.

TABLE 3

The properties of episomal gal+ transductants derived from rec+ and rec- recipients

Recipient	Total colonies tested	Transmissible		Nontransmissible†		Integrated gal
		Entire gal	Deleted gal*	Entire gal	Deleted gal	
W3623 rec+	400	154	16	0	0	230
W3623 rec-	405	351	29	24	1	0
JE3264 rec+	100	50	0	0	0	50

* Mutants with deletion mutations in *galK* cistron on F_8. They complemented *galE* and *galT* mutants but not *galK* mutants upon transfer to the appropriate recipients.

† Transfer-defective mutants of F_8. Their gal+ transfer was restored by introduction of R_{100-1}.

F_8 carries the entire galactose operon, three cistrons in the order, operator–epimerase (*galE*)–transferase(*galT*)–kinase(*galK*). The recipient W3623 was a *galT*- mutant. All Gal+ transductants having transmissible F*gal* complemented the *galE* mutant, W3995 *gal-22*, but did not complement various kinds of *galK* mutants upon transfer of F*gal* to the appropriate recipients. Among Gal+ transductants of JE3264 *galT-1 galK-2*, there formed no *galE* mutant of F *gal*. Thus, no mutant which lacks the operator side of the galactose operon was observed. These results indicate that these mutants were formed by deletions of the distal end of the galactose operon.

The nontransmissible Gal+ transductants were examined to determine if they contained defects in the cistrons involved in genetic transfer. If so, it was expected that the strains would recover transfer ability for *gal* genes when R_{100-1} was introduced into them (OHTSUBO, NISHIMURA and HIROTA 1970). None of the nontransmissible Gal+ transductants derived from the rec+ recipient recovered *gal*+ transfer ability when R_{100-1} was introduced. On the other hand, all of those derived from the rec- recipient recovered *gal*+ transfer ability when infected with R_{100-1}. Thus the nontransmissible Gal+ transductants derived from the rec- recipient are presumed to have transfer-defective F *gal* with deletions in F. In the rec+ recipient, the *gal*+ genes were presumably integrated into the chromosome and all or a great part of F was lost or excluded. The nontransmissible Gal+ transductants obtained from the rec+ recipient were not cured of Gal+ properties by treatment with acridine orange while the transmissible Gal+ transductants were efficiently cured. (*gal* genes of all Gal+ transductants obtained from the rec- recipient were not cured. Even the genes on wild F_8 transferred into this recA- recipient strain were not cured. These nontransmissible or transmissible *gal* genes transferred into a Gal- rec+ recipient with or without the helper R_{100-1} were cured by acridine. Thus, these F *gal* in the rec- recipient were in an autonomous state but resistant to acridine curing.)

From 200 Cm^R transductants of R_{100-1} derived either from rec+ or rec- recipients, no nontransmissible mutant of R was found. One transductant derived from the rec+ recipient was tetracycline-sensitive (Tc^S).

Deletion mapping of the galK *cistron:* The twenty-nine transductants harboring transmissible F *gal* with deletions in the *galK* cistron were analyzed by

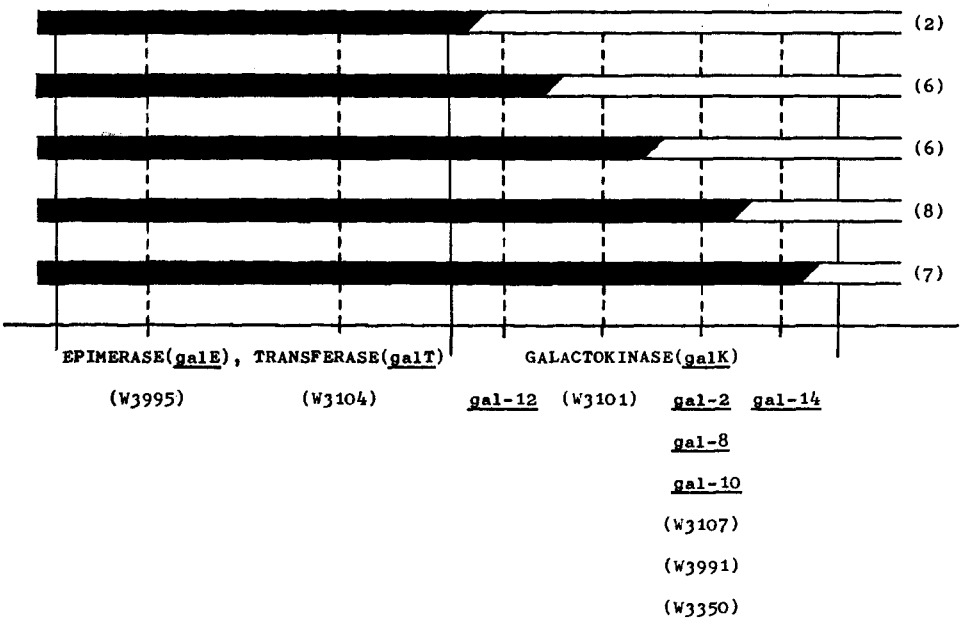

FIGURE 1.—Deletion map of the *galK* mutations. This map was constructed by the method of overlapping deletions. Open spaces show deleted regions of *gal* genes. *gal* numbers show gal⁻ mutations in *galK* and W numbers show the strains originally classified as *galE* or *galT* mutants. Numbers in ( ) show the number of independently isolated mutants of F_8 with similar deletions in *galK* (see Footnote to Table 3).

mating of a number of *gal*⁻ mutants by spot tests. It is possible to arrange the *galK* mutants in such a way that those recombining with deletion mutants of F *gal* form an unbroken sequence (Figure 1). The order obtained above for *galK* mutation sites agrees with the results of analyses by λdg (DAVISON, FRAME and BISHOP 1967).

Among mutants originally classified as *galT* mutants, there are some which are not complemented by F *gal* with deletions in the *galK* cistron and which map in *galK* (Figure 1). For instance, W3101 (*galT-1*) and W3991 (*galT-1*) map at different sites on *galK*. It is known that *galK* mutants tend to arise spontaneously in *galE* and *galT* stocks due to greater viability (KALCKAR, KURAHASHI and JORDAN 1959; YARMOLINSKY *et al.* 1959). The *galT* mutants such as W3101, W3991, and W3107 may be such double mutants. In fact, one of the mutant sites in W3350 was mapped in *galK*, presumably the *gal-2* site. A triply defective mutant, W3109 which is known to be deficient in three enzymes of the galactose operon and to be a single-site mutation (*gal-9*) (LEDERBERG 1960), forms Gal⁺ recombinants with all of the deleted F *gal* mutants.

Deletion mapping of the cistrons involved in genetic transfer: The thirty-one transductants harboring transfer-defective F_8 were analyzed by complementation tests with transfer-defective R mutants. They did not always recover *gal*⁺ transfer ability when a set of R mutants were transduced by phage P1*kc* and could be

TABLE 4

Complementation analysis of transfer-defective mutants of F_8*

Group	Number of mutants	Transfer-defective R factor introduced†					
		$R_{100-300}$	R_{100-67}	R_{100-73}	R_{100-75}	R_{100-69}	R_{100-70}
1‡	15	—	+	+	+	+	+
2	9	—	—	+	+	+	+
3	4	—	—	—	+	+	+
4	1	—	—	—	—	+	+
5	2	—	—	—	—	—	+

* A set of transfer-defective R factors were transduced by phage P1*kc* into the cells harboring transfer-defective F_8 mutants (see MATERIALS AND METHODS). Recovery of transfer of F *gal* shows complementation occurred between defective F and defective R factors and designated as +.

† $R_{100-300}$, R_{100-67}, R_{100-73}, R_{100-75}, R_{100-69}, and R_{100-70} are mutants having mutations in the *A*, *B*, *C*, *D*, *E*, and *F* cistrons, respectively, which are involved in genetic transfer and analyzed in the preceding paper (OHTSUBO, NISHIMURA and HIROTA 1970).

‡ One mutant in group 1 contains a deletion in the *galK* cistron.

classified into five groups as shown in Table 4. Among F_8 mutants of group 1, one mutant contains a deletion extending from the *A* cistron of the F-prime and into the *galK* cistron, between *gal-14* and *gal-2*. Thus, defects in one or more transfer cistrons of these F_8 mutants can be explained as being due to the deletions. By the method of overlapping deletions, a map of F_8 was constructed (Figure 2).

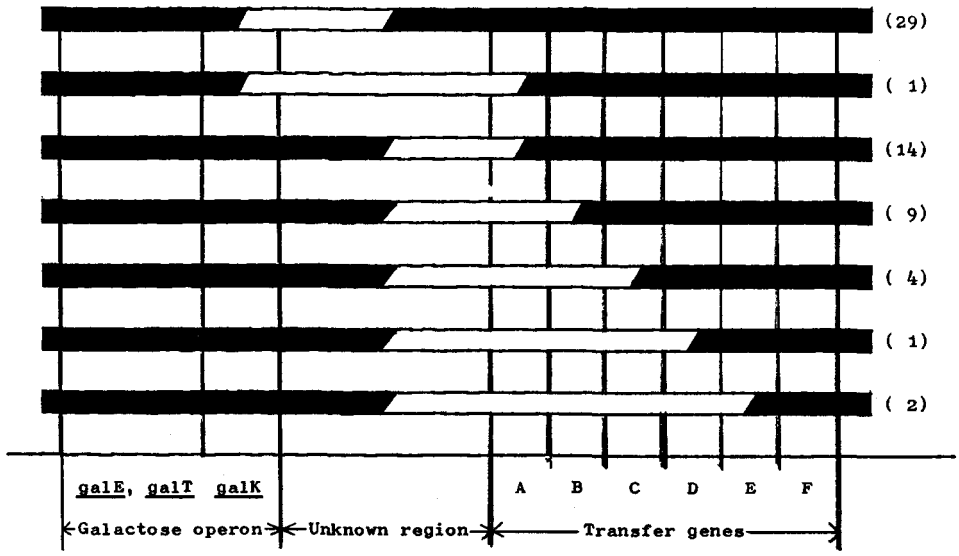

FIGURE 2.—Deletion map of transfer genes on F_8. This map was based on the data in Table 4 and constructed with galactose operon by the method of overlapping deletions. Open spaces show deleted regions on the F_8 chromosome. The numbers in ( ) show the number of independently isolated deletion mutants.

TABLE 5

Male properties associated with deletions in transfer cistron

Group	Cistron(s) deleted	Male-specific phage sensitivity to:						Growth inhibition to phage τ	Number of mutants
		M12	MS2	f2	f1	Qβ	ZD		
1	*A*	S	S	S	S	S	S	+	15
2	*A, B*	R	R	R	S	S	S	+	9
3	*A, B, C*	R	R	R	S	S	S	+	1
		R	R	R	R	R	R	+	3
4	*A, B, C, D*	R	R	R	R	R	R	+	1
5	*A, B, C, D, E*	R	R	R	R	R	R	+	2
F_8+	Wild	S	S	S	S	S	S	+	..

Symbols S and R signify sensitive and resistant to male-specific phage, respectively. All the strains having deletion mutants of F *gal* inhibit the growth of phage τ.

Male properties of deletion mutants of F_8: It is of interset to know which male properties are defective in deletion mutants of F *gal* that have lost one or more cistrons involved in conjugation, since mutants of F and R factors with mutations in each cistron have characteristic properties (OHTSUBO, NISHIMURA and HIROTA 1970). As summarized in Table 5, they retained all or a part of the male properties analyzed.

DISCUSSION

Transfer-defective mutants of F_8 could be isolated among Gal+ transductants after transduction with phage P1*kc*. Such deletion mutants were isolated only in a rec- recipient. However, mutants of F *gal* with deletions in the *galK* cistron could be isolated at the same frequency among Gal+ transductants harboring F *gal* derived both from rec+ and rec- recipients.

It should be noted that the transduction frequency of F*gal* in the rec- recipient was much lower than in the rec+ recipient. This difference may be related to a lower efficiency of circle formation in the rec-, since some sorts of autonomously replicating F factors have been shown to have circular DNA forms (HICKSON, ROTH and HELINSKI 1967; FREIFELDER 1968), and DNA in transducing particles is linear (IKEDA and TOMIZAWA 1965). Thus the transducing DNA molecule probably has to circularlize in the recipient before it can establish itself as an autonomously replicating unit. This circularization may be effected by recombination events controlled by the rec system. An R factor which lacks homology with the host chromosome was transduced at a similar frequency to rec+ and rec- recipients. Therefore, the failure to isolate transfer-defective F_8 mutants in the rec+ recipient may be due to the fact that mutants with deletions in part of an F moiety are unstable in the rec+ and their homologous *gal* genes alone tend to be integrated into host chromosome.

Deletion mutants of an R factor were not readily isolated by transduction with phage P1*kc* and a higher transduction frequency was observed with R than with

F_8. These differences might result from a difference in the number of DNA copies (IKEDA and TOMIZAWA 1965), or from different degrees of linkage of the selective markers with the genes necessary for autonomous replication.

It is of interest that in the various kinds of deletion mutants obtained by transduction of F_8 into a rec$^-$ recipient, the two ends of transduced DNA which has *gal* genes on one end and the cistrons controlling conjugation on another end were not fixed. This fact raises the possibility that the linear DNA molecule of the transducing fragment may circularize by illegitimate crossing over at the ends of the deletion. Illegitimate crossing over, producing deletions, has been shown to occur in rec$^-$ cells (FRANKLIN 1967; INSELBURG 1967).

The order of cistrons involved in genetic transfer has been determined by the method of overlapping deletions; they can be arranged on F_8 with the galactose genes in the following order: *gal* (operator-epimerase-transferase-kinase)-*A*-*B*-*C*-*D*-*E*-*F*. One group of cistrons (*A* and *B*) containing mutations that do not affect production of specific pili (*pili*$^+$) are contiguous; another group of cistrons (*C*, *D*, *E*, and *F*) contain mutations which affect F-pili production (*pili*$^-$) and these cistrons are also contiguous.

The male properties associated with deletion mutations show that even when all or a part of cistrons *A* and *B* is deleted, the F-pilus is formed. This confirms the previous conclusion that cistrons *A* and *B* are not concerned with the piliation specific to F and R factors, except for the possibility that they control some minor components of the pilus (OHTSUBO, NISHIMURA and HIROTA 1970). Analysis of the mutations in cistron *C* had shown the same peculiarity, in that some mutants containing these mutations were *pili*$^+$ and others were *pili*$^-$. That such phenotypically similar mutants were obtained as deletion mutations may suggest the presence of subunits in cistron *C* which probably control piliation. The *pili*$^+$ mutants having deletions for all of cistron *A* and at the same time, for a part or all of cistron *B* in which mutants are resistant to some, but sensitive to other, male-specific RNA phages, show the same phenotype as mutants with mutations in cistron *B*. As for cistrons *D*, *E*, and *F* it was also confirmed that they were probably genes controlling pili formation, as mutants with deletions in these cistrons did not produce F-pili.

The author is grateful to DRS. YUKINORI HIROTA and YUKINOBU NISHIMURA for their guidance throughout this work. The author also wishes to express his thanks to PROFESSOR JUN-ICHI TOMIZAWA for his encouragement during the course of this work, and to DRS. HARUO OZEKI and YOSHINOBU SUGINO for helpful discussions.

SUMMARY

Phage P1*kc*-mediated transduction of galactose genes of an F-prime, F_8, into a rec$^-$ gal$^-$ recipient could efficiently select autonomous deletion mutants of F_8 having defects in one or more cistrons involved in genetic transfer and the galactokinase cistron of the galactose operon. By the method of overlapping deletions, these cistrons were found to form a cluster and were arranged on F_8 with the galactose genes in the following order: *gal*(operator-epimerase-transferase-kinase)-*A*-*B*-*C*-*D*-*E*-*F*.

LITERATURE CITED

CLOWES, R. C. and E. E. M. MOODY, 1966 Chromosomal transfer from "recombination-deficient" strains of *Escherichia coli* K-12. Genetics **53**: 717–726.

DAVISON, J., R. FRAME and J. BISHOP, 1967 A partial deletion map of the galactose operon in *E. coli* K12. Genet. Res. **10**: 107–116.

DAVIS, B. D. and E. S. MINGIOLI, 1950 Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**: 17–28.

EGAWA, R. and Y. HIROTA, 1962 Inhibition of fertility by multiple drug-resistance factor (R) in *Escherichia coli* K-12. Japan. J. Genet. **37**: 66–69.

FRANKLIN, N. C., 1967 Extraordinary recombinational events in *Escherichia coli*. Their independence of the *rec*+ function. Genetics **55**: 699–707.

FRÉDÉRICQ, P., 1965 Genetics of colicinogenic factors. Zbl. Bakteriol., I. Abt. Orig. **196**: 142–151.

FREIFELDER, D., 1968 Studies on *Escherichia coli* sex factors. III. Covalently closed F′ *lac* DNA molecules. J. Mol. Biol. **34**: 31–38.

HICKSON, F. T., T. F. ROTH and D. R. HELINSKI, 1967 Circular DNA forms of a bacterial sex factor. Proc. Natl. Acad. Sci. U. S. **58**: 1731–1738.

HIROTA, Y. and P. H. A. SNEATH, 1961 F′ and F-mediated transduction in *Escherichia coli* K12. Japan. J. Genet. **36**: 307–318.

IKEDA, H. and J. TOMIZAWA, 1965 Transducing fragments in generalized transduction by phage P1. I. Molecular origin of the fragments. J. Mol. Biol. **14**: 85–109.

INSELBURG, J., 1967 Formation of deletion mutations in recombination-deficient mutants of *Escherichia coli*. J. Bacteriol. **94**: 1266–1267.

KALCKAR, H. M., K. KURAHASHI and E. JORDAN, 1959 Hereditary defects in galactose metabolism in *Escherichia coli* mutants, I. Determination of enzyme activities. Proc. Natl. Acad. Sci. U. S. **45**: 1776–1786.

LEDERBERG, E. M., 1960 Genetic and functional aspects of galactose metabolism in *Escherichia coli* K-12. Microbial Genetics, Symposium Soc. Gen. Microbiol. **10**: 115–131.

LENNOX, E. S., 1955 Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**: 190–206.

MORSE, M. L., E. M. LEDERBERG and J. LEDERBERG, 1956a Transduction in *Escherichia coli* K-12. Genetics **41**: 142–156. ——, 1956b Transductional heterogenotes in *Escherichia coli*. Genetics **41**: 758–779.

OGAWA, H., K. SHIMADA and J. TOMIZAWA, 1968 Studies on radiation-sensitive mutants of *E. coli*. I. Mutants defective in the repair synthesis. Molec. Gen. Genetics **101**: 227–244.

OHTSUBO, E., Y. NISHIMURA and Y. HIROTA, 1970 Transfer-defective mutants of sex factors in *Escherichia coli*. I. Defective mutants and complementation analysis. Genetics **64**: 173–188.

SUGINO, Y. and Y. HIROTA, 1962 Conjugal fertility associated with resistance factor R in *Escherichia coli*. J. Bacteriol. **84**: 902–910.

TAYLOR, A. L. and C. D. TROTTER, 1967 Revised linkage map of *Escherichia coli*. Bacteriol. Rev. **31**: 332–353.

WATANABE, T. and T. FUKASAWA, 1961 Episome-mediated transfer of drug resistance in Enterobacteriaceae. III. Transduction of resistance factors. J. Bacteriol. **82**: 202–209.

WILKINS, B. M., 1969 Chromosome transfer from F-*lac*+ strains of *Escherichia coli* K-12 mutant at *recA*, *recB*, or *recC*. J. Bacteriol. **98**: 599–604.

YARMOLINSKY, M. B., H. WIESMEYER, H. M. KALCKAR and E. JORDAN, 1959 Hereditary defects in galactose metabolism in *Escherichia coli* mutants, II. Galactose-induced sensitivity. Proc. Natl. Acad. Sci. U. S. **45**: 1786–1791.