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CHROMOSOMAL LOCATION OF THYMINE AND ARGININE GENES IN *ESCHERICHIA COLI* AND AN F′ INCORPORATING THEM

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ABSTRACT

ISHIBASHI, MASAHIDE (Osaka University, Osaka, Japan), YOSHINOBU SUGINO, AND YUKINORI HIROTA. Chromosomal location of thymine and arginine genes in *Escherichia coli* and an F′ incorporating them. J. Bacteriol. 87:554–561. 1964.—The gene responsible for thymine requirement or independence in *Escherichia coli* K-12 is located on the chromosome, near Arg_2 (arginine) and between *Sm* (streptomycin) and *Ade* (adenine). A new F′ (called F_{15}) carrying the *Thy* (thymine) gene as well as the Arg_2 gene was discovered. The Thy^+ (thymine independent) gene on F_{15} relieves the defect of all Thy^- (thymine requiring) mutants of *E. coli* examined, such as *E. coli* 15 T^-, B3, and Thy^- mutants obtained by aminopterine treatment. It can also be transferred to Thy^- mutants of *Salmonella typhimurium* and *Serratia marcescens*, converting them to Thy^+.

A "thymineless" (Thy^-) mutant is a mutant that cannot grow in the absence of an external supply of thymine or thymidine (Cohen and Barner, 1954; Brenner and Smith, 1959; Kaudewitz, 1959). Growth in aminopterine-containing medium permits ready isolation of thymineless mutants of *Escherichia coli* including strain K-12 (Okada, Yanagisawa, and Ryan, 1960; Okada, 1962), but the chromosomal locus of these mutations has not yet been determined.

In the present paper, we report the determination of the locus of this thymine gene on the chromosome and the discovery of an F′ carrying the Thy^+ (thymine independent) gene and another chromosomal gene Arg_2^+ (arginine independent). A preliminary report of this work was published (Ishibashi et al., Japan. J. Genetics, *in press*).

MATERIALS AND METHODS

Genetic markers. Nutritional requirements ($^-$ indicates dependence; $^+$, independence) are shown by the following symbols: *Nia*, niacin; *M*, methionine; *Thr*, threonine; *L*, leucine; *Pur*, purine (optimal growth with thiamine and adenine or guanine); *Try*, tryptophan; *His*, histidine; *Ade*, adenine; *Ser/Gly* serine or glycine; *Arg*, arginine; and *Thy*, thymine. (The different suffixes indicate different isolations.)

Fermentation markers ($^-$ indicates non-fermentation; $^+$, fermentation) include: *Ara*, arabinose; *Gal*, galactose; *Lac*, lactose; *Mal*, maltose; *Mtl*, mannitol; and *Xyl*, xylose.

Sm^r and Sm^s indicate resistance and sensitivity to streptomycin, respectively.

F factor. The maleness, or donor capacity (♂), of genetic markers of *E. coli* is determined by the presence of an episome called an F factor (Lederberg, Cavalli, and Lederberg, 1952; Hayes, 1953; Jacob, Schaeffer, and Wollman, 1960). Female or recipient (F^-) lacks this factor.

Males (♂) include F^+, Hfr, and F'^+.

F^+ indicates infective wild-type males. Hfr are males showing a high frequency of recombination. Hfr_1 transfers the chromosome in the order *Pur Lac L Ara Thr M Mtl Xyl Mal Sm* (Cook and Lederberg, 1962).

F′ and F-duction. The F factor sometimes forms a stable complex, called F′, with a fragment of the chromosome. The F′ is characterized by infective transmission of high fertility; i.e., when these agents infect an F^- cell, the recipient cell is converted to a state of high male fertility, and this state may further be transmitted to other F^- cells (Adelberg and Burns, 1959). This phenomenon is interpreted as due to the affinity of the incorporated fragment of the chromosome for a homologous region of the host chromosome. Different F′ are distinguished by numeral suffixes added to F. Some F′ carry known chromosomal markers; e.g., F_8 carries the *Gal* locus, and F_{13} carries the chromosomal segment including the *Pur* and *Lac* genes (Jacob and Adelberg, 1959; Hirota and Sneath, 1961). Such genes are transferred from cell to cell at high

frequency in association with the F factor, independently of the chromosome. This infective transmission of genes by incorporation in the F factor is called F-duction, and the cell that has received such an F′ is called an F-ductant.

Bacteria. The strains of *E. coli* K-12 used are listed in Table 1. *Thy*⁻ mutants of *E. coli* K-12, *Salmonella typhimurium* LT-2, and *Serratia marcescens* were obtained by treatment with aminopterine. *E. coli* 15 T⁻ and *E. coli* B3 were also used as examples of *Thy*⁻ mutants obtained by other means.

Media. Nutrient broth contained 10 g of polypeptone, 10 g of meat extract, and 2 g of NaCl in 1 liter of water, and was adjusted to pH 7.0 with NaOH. For growth of thymineless bacteria, 50 μg of thymine (or sometimes 80 μg of thymidine) were usually added per ml. Nutrient agar was prepared by adding 1.5% agar to nutrient broth. Davis minimal medium (DM-Glu), minimal Eosin Methylene Blue (EMS) medium without succinate (EM), and complete Eosin Methylene Blue (EMB) medium were prepared as described by Lederberg (1950). For selection of some classes of recombinants, glucose in Davis minimal medium was replaced by lactose (DM-Lac), galactose (DM-Gal), etc. Nutrients or antibiotics were added as indicated.

Isolation of thymineless mutants by treatment with aminopterine. The method of Okada, Homma, and Sonohara (1962) was followed.

Acridine treatment. The method of Hirota (1960) was followed.

F-duction. Cultures of donor and recipient bacteria in the exponential growth phase (ca. 2×10^8 to 3×10^8 cells per ml) in nutrient broth were mixed, and, after standing for 1 hr at 37 C, the mixture was plated on agar where only recipient bacteria could form colonies. These plates were replica-plated to test markers transferred from the donor (Lederberg and Lederberg, 1952). Control plates were seeded with recipient bacteria alone, and treated similarly.

Spot test for transfer of markers. A drop of the donor culture was spotted on a dried film of the recipient culture on a selective agar medium. The selection was so arranged that growth occurred only when there was transfer of test markers.

Cross between Hfr and F⁻. The procedure for mixing of donor and recipient bacteria was similar to that for F-duction. The mixtures were plated on selective media, where only recombinants could form colonies. Recombinant colonies were picked at random and purified, and unselected markers were scored by replica plating.

Interrupted mating. In this experiment, cultures of donor and recipient bacteria were used in the exponential growth phase (2×10^8 to 3×10^8 cells per ml). Donor culture (2 ml) and recipient bacteria culture (10 ml) were mixed at 37 C in a flask and incubated. Samples were taken at intervals, diluted 200 times with ice-cold Davis

TABLE 1. *Strains of Escherichia coli K-12 used*

Strain	Genotype*
W6	F^+M^-
W1895	Hfr_1M^-
W3555	F^-His^-
W4183	$F^-Arg_1^-Sm^r$
W4354	F^-M^-
W4573	$F^-Lac^-Gal^-Ara^-Xyl^-Mtl^-Mal^-Sm^r$
W4520	$F_8^+M^-$
W4580	$F_{15}^+M^-$
JE346	$F^-Pur^-Try^-Lac^-Gal^-Ara^-Xyl^-Mtl^-Mal^-Sm^r$
JE666	$F^-Mal_5^-Thy_4^-$
JE850	Thy_5^- derivative of JE346
JE888	Thy_6^- derivative of W1895
JE1021	$F^-Thy_{14}^-Arg_2^-Sm^rGal^-$
JE1025	$F^-Thy_{16}^-Arg_2^-Sm^rGal^-$
JE1040	$F^-Thy_{16}^-Arg_2^-Ade^-Gal^-$
JE1063	$F^-Thy_{18}^-Nia^-Gal^-$
JE1064	$F^-Thy_{17}^-Ser/Gly^-$

* Only relevant markers are shown as genotype.

minimal medium minus glucose, and agitated in a homogenizer. They were then plated on various selective media. *Thy*$^+$*Sm*^{r} selection was made on DM-Glu medium plus 100 μg of streptomycin, 50 μg of Casamino Acids, 50 μg of arginine, and 20 μg of adenine per ml. *Ade*$^+$ *Sm*^{r} selection was made on DM-Glu medium plus 100 μg of streptomycin, 50 μg of Casamino Acids, 50 μg of arginine, and 50 μg of thymine per ml.

RESULTS

F-duction of the Thy gene. Various *E. coli* K-12 strains with F′ isolated by Y. Hirota at Stanford University were tested to determine whether the F′ carried the *Thy* gene. Of the F′$^+$ strains screened, only W4580 carrying F_{15}, which transfers the *Gal* locus at relatively high frequency (ca. 10^{-3}), was found to transfer the *Thy* gene in association with the F factor. The results of a typical experiment are shown in Table 2. The *Thy*$^+$ gene was transferred at high frequency from W4580 to JE666, and all bacteria that received the *Thy*$^+$ gene also received the F factor.

Ordinary F$^+$ (and F$^-$) strains did not transfer the *Thy*$^+$ gene at such a high efficiency. The *Thy*$^+$ clones obtained in the F-duction experiment could transfer the *Thy*$^+$ character further at high frequency to other *Thy*$^-$ mutants.

All the *Thy*$^-$ mutants obtained by Okada's method, and listed in Table 1, were found to be converted at high frequency to *Thy*$^+$ upon contact with W4580.

TABLE 2. *F-duction of the Thy*$^+$ *gene, from W4580 (*$F_{15}$$^+$*M*$^-$*Sm*^{s}*) to JE666 (F*$^-$*Thy*$^-$*Mal*$^-$*)**

Strain mixed with JE666	Total no. of colonies examined	*Thy*$^+$		*Thy*$^-$	
		♂	♀	♂	♀
W4580 ($F_{15}$$^+$*M*$^-$)	267	42	0	0	225
W4520 ($F_8$$^+$*M*$^-$)	140	0	0	73	67
W6(F$^+$*M*$^-$)	303	0	0	72	231
W4354(F$^-$*M*$^-$)	197	0	0	0	197
Control (JE666 alone)	411	0	0	0	411

* Equal volumes of donor and recipient cultures were mixed. The mixed cultures were plated on Davis minimal medium supplemented with 50 μg of thymine per ml. Methionine-requiring bacteria form no colonies on this medium. The colonies formed were scored for *Thy*$^+$ or *Thy*$^-$ by replica plating on Davis minimal medium. The fertility status, ♂ or ♀, was determined by replica plating on EM-maltose agar supplemented with thymine seeded with W4354.

TABLE 3. *Disinfection of* F_{15} *with acridine orange from JE666 (F*$^-$*Thy*$^-$*) infected with* F_{15}*

Treatment	Total no. of colonies examined	*Thy*$^+$		*Thy*$^-$	
		♂	♀	♂	♀
Acridine-treated	1,071	24	18	0	1,029
Control without acridine treatment	1,632	1,632	0	0	0

* A *Thy*$^+$ colony obtained by transfer of F_{15} from W4580($F_{15}$$^+$*M*$^-$) to JE666 (F$^-$*Thy*$^-$*Mal*$^-$) was purified by single-colony isolation and used for the experiment. Acridine treatment was performed in Penassay broth supplemented with 50 μg of thymine per ml. The treated culture was plated out, and the character of individual colonies was scored by the replica plating method. The fertility status (♂ or ♀) was checked by transfer of the *M*$^+$ gene to W4354 (F$^-$*M*$^-$).

Elimination of F_{15} *with acridine orange.* A *Thy*$^+$ clone obtained by F-duction from W4580 to JE666 was treated with acridine orange, and tested for loss of male and *Thy*$^+$ characters (Table 3). As shown in the table, the *Thy*$^+$ character was lost simultaneously with male fertility. This indicates that JE666$F_{15}$$^+$ are heterogenetic with respect to the *Thy* gene. The chromosomal allele is *Thy*$^-$, whereas the *Thy*$^+$ allele is carried by the F′ factor.

Upon acridine treatment of W4580, male fertility (as determined by the transfer of the *Gal*$^+$ character to W4573) and the high efficiency of transmission of the *Thy*$^+$ character were simultaneously lost. No *Thy*$^-$ clones were produced, however, indicating that W4580 were homogenotic with respect to the *Thy* locus, and still carried the *Thy*$^+$ gene on the chromosome after elimination of F_{15} in which the (exogenotic) *Thy*$^+$ gene was contained.

F$^-$*Thy*$^+$ bacteria also gave no *Thy*$^-$ clones upon acridine treatment.

Transfer of F_{15} *to thymineless mutants of strains of E. coli other than K-12.*

E. coli 15 T$^-$ (Cohen and Barner, 1954) and B3 (Brenner and Smith, 1959) could be converted to thymine independence by mixed culture with W4580. In one experiment, about 1% of the 15 T$^-$ cells became *Thy*$^+$ after mixed culture

TABLE 4. *Joint F-duction of Thy^+ and Arg_2^+ from W4580 ($F_{15}^+M^-Sm^s$) to JE1025 ($F^-Thy^-Arg_2^-Gal^-Sm^r$)**

Strain	Total no. of colonies examined	Thy^+Arg^+ ♂	Thy^-Arg^- ♀
Mixture of JE1025 and W4580	1,090	204	886
Control (JE1025 alone)	764	0	764

* Donor and recipient cultures were mixed in the ratio of 20:1 (v/v). Samples were plated on nutrient agar containing 100 μg of streptomycin per ml, which eliminated the donor bacteria. Procedures for scoring for nutritional requirements and fertility were essentially similar to those used in Table 2.

with W4580 for 1 hr, while about 10^{-3} B3 cells became Thy^+ under similar conditions.

Such thymine-independent cells segregated thymineless bacteria, and could transfer F_{15} back to Thy^- mutants of K-12.

Transfer of F_{15} to other species. Thymineless mutants of *Serratia marcescens* and *Salmonella typhimurium* LT-2 were obtained by aminopterine treatment. These mutants were mixed with W4580, and Thy^+ progeny could be selected by plating on appropriate selective medium. These thymine-independent progeny were similar to the *Serratia* or *Salmonella* parent in other respects, and could transfer F_{15} back to Thy^- mutants of *E. coli* K-12.

Joint F-duction of Thy and Arg_2 genes. The Arg_2 gene (in Lederberg's notation) is reported to lie between *Sm* and *Try* (Richter, 1959; cited in Hirota and Sneath, 1961). Our data of crosses with several Hfr strains confirm this result.

Results of a cross (described in the subsequent section) suggested that the *Thy* locus lay between *Sm* and *Try*. Several markers in this region, including *Sm*, *Ade*, *Ser/Gly*, Arg_1, *Nia*, Arg_2, and *His*, were screened for joint F-duction with the *Thy* gene. Only Arg_2 was found to be jointly transmitted with the *Thy* gene in association with the F factor (Table 4). The Thy^+ and Arg_2^+ character of the clones obtained by F-duction to a $Thy^-Arg_2^-$ strain were simultaneously lost upon elimination of F with acridine orange (Table 5).

Chromosomal mapping of the Thy gene by use of crosses between Hfr and F^-. The results of reciprocal crosses between Hfr_1 strains and F^- strains are shown in Table 6. The first cross involved a Thy^+Hfr_1 strain and Thy^-F^- strain, while the second cross involved a Thy^-Hfr_1 and Thy^+F^- strain. The two Hfr and two F^- strains were isogenic except for the *Thy* gene. M^+Gal^+ recombinant colonies, which occurred at a rate of about 10^{-4} per Hfr cell, were picked at random, purified, and scored for unselected markers. The allele of the *Thy* gene derived from the Hfr parent was transmitted to the M^+Gal^+ recombinants at the same frequency in both crosses; this indicates that the *Thy* gene is not cytoplasmic but has a definite chromosomal locus. In Table 7, the frequency of the Thy^+ gene from the Hfr_1 parent (W1895) among recombinants selected by various markers is shown as well as the frequency of other unselected markers. In Table 6, the occurrence of male markers shows a gradient which decreases from the proximal marker *Pur* to the contraselective marker *M*, and then increases to the distal selective marker *Gal*. The values for *Thy* place it either between *Lac* and *Ara*, or between *Sm* and *Try*. However, the former possibility is excluded, because the results in Table 7 show that there is no linkage of *Thy* to *Ara*, but a linkage of *Thy* to *Sm*.

TABLE 5. *Disinfection of F_{15} with acridine orange from JE1021 ($F^-Thy^-Arg_2^-$) infected with F_{15}**

Treatment	Total no. of colonies examined	Thy^+Arg^+		Thy^-Arg^-		Thy^+Arg^+		Thy^+Arg^-	
		♂	♀	♂	♀	♂	♀	♂	♀
Acridine-treated	390	13	0	0	371	0	3	1	2
Control without acridine treatment	812	809	0	0	0	1	0	1	1

* Three Thy^+Arg^+ clones obtained by transfer of F_{15} from W4580 ($F_{15}^+M^-$) to JE1021 ($F^-Thy^-Arg_2^-Gal^-Sm^r$) were used. The sum of data from the three independent experiments is shown. Procedures used were similar to those in Table 3.

TABLE 6. *Frequency of occurrence of unselected male markers in Gal^+M^+ recombinants from crosses (1) $Hfr_1Thy^+M^-Gal^+$ (W1895) × $F^-Thy^-M^+Gal^-$ (JE850) and (2) $Hfr_1Thy^-M^-Gal^+$ (JE888) × $F^-Thy^+M^+Gal^-$ (JE346)**

Cross	No. of colonies examined	Percentage of occurrence of male markers										
		Thy	*Pur*	*Lac*	*Ara*	*M*	*Mtl*	*Xyl*	*Mal*	*Sm*	*Try*	*Gal*
1	100	59	78	66	48	0	25	27	38	39	79	100
2	110	57	91	77	56	0	21	24	40	41	93	100

* W1895: $Hfr_1Thy^+Pur^+Lac^+Ara^+M^-Mtl^+Xyl^+Mal^+Sm^sTry^+Gal^+$. JE850: $F^-Thy^-Pur^-Lac^-Ara^-M^+Mtl^-Xyl^-Mal^-Sm^rTry^-Gal^-$. JE888: $Hfr_1Thy^-Pur^+Lac^+Ara^+M^-Mtl^+Xyl^+Mal^+Sm^sTry^+Gal^+$. JE346: $F^-Thy^+Pur^-Lac^-Ara^-M^+Mtl^-Xyl^-Mal^-Sm^rTry^-Gal^-$. Equal volumes of donor and recipient cultures were mixed. After standing for 3 hr at 37 C, the mixture was plated on DM-Gal agar supplemented with 50 μg of adenine, 5 μg of thiamine, 50 μg of tryptophan, and 50 μg of thymine per ml. The M^+Gal^+ recombinant colonies formed were purified and scored for unselected markers by replica plating. The order of markers in this table, except for *Thy*, is as in a standard chromosome map (Jacob and Wollman, 1961).

TABLE 7. *Frequency of unselected male markers transferred to recombinants selected by various markers in the cross W1895 × JE850**

Selection	No. of colonies examined	Percentage of occurrence of male markers										
		Thy	*Pur*	*Lac*	*Ara*	*M*	*Mtl*	*Xyl*	*Mal*	*Sm*	*Try*	*Gal*
Ara^+Sm^r	56	0	32	71	100	9	0	0	0	0	0	0
Xyl^+Sm^r	56	2	7	41	48	57	75	100	19	0	2	0
Gal^+Sm^r	56	16	77	66	39	16	4	0	0	0	79	100
Gal^+M^+	60	57	82	65	48	0	15	18	30	25	88	100

* W1895: $Hfr_1Thy^+Pur^+Lac^+Ara^+M^-Mtl^+Xyl^+Mal^+Sm^sTry^+Gal^+$. JE850: $F^-Thy^-Pur^-Lac^-Ara^-M^+Mtl^-Xyl^-Mal^-Sm^rTry^-Gal^-$. The conditions of mating were similar to those for Table 6, except that the ratio of donor to recipient cultures was 1:5. Ara^+Sm^r, Xyl^+Sm^r, or Gal^+Sm^r recombinants were selected on EMB agar containing the respective sugars and supplemented with 100 μg of streptomycin and 50 μg of thymine per ml. Gal^+M^+ recombinants were selected as in Table 6. Other procedures used were similar to those for Table 6.

Thus, the results in Tables 6 and 7 indicate that the *Thy* locus lies between *Sm* and *Try*, near *Sm*.

To determine the *Thy* locus more precisely, a Thy^-F^- strain carrying the Ade^- and Arg_2^- mutations (JE1040) was used as partner in a cross with Hfr_1 (W1895). [Ade^- mutation used here, originally introduced by A. Cook (*personal communication*), probably corresponds to that cited by Taylor and Adelberg (1960). From the data of crosses with several Hfr strains, both Arg_2 and *Ade* appear to lie between *Sm* and *Try*, with the order *Sm* Arg_2 *Ade Try*.] Ade^+ Sm^rM^+ and Gal^+Sm^r recombinants were selected, and the unselected markers were scored. The results indicate that the *Thy* locus is closely linked to the Arg_2 locus (Table 8). The order of these loci is probably *Sm* (*Thy* Arg_2) *Ade Gal*, although the order *Thy* Arg_2 is only tentative.

Figure 1 shows the location of the *Thy* and Arg_2 genes on the circular chromosome map of *E. coli*.

Interrupted-mating experiments. Figure 2 gives the kinetics of transfer of markers from W4580 to JE1040, as shown by interrupted-mating experiments. W4580 begins to transfer the Thy^+ gene about 5 min after the initiation of mating. The chromosomal Ade^+ gene is transferred about 10 min after the Thy^+ gene. The Thy^+ colonies were picked up and purified, and scored for Arg_2^+, Ade^+, and for high frequency transmissibility of the Thy^+ gene. Figure 3 shows that the Arg_2^+ gene closely follows the Thy^+ gene, and that the ability to transfer further the Thy^+ gene at high frequency (or F in association with the Thy^+ gene) closely follows the Arg_2^+ gene. All the clones were Ade^-. Some of the clones that did not transfer the Thy^+ gene at high frequency

TABLE 8. *Linkage of Thy with Arg_2 and Ade in the cross W1895 × JE1040**

Recombinants	*M*	*Sm*	*Thy*	Arg_2	*Ade*	*Gal*	No. of recombinants†
$M^+Sm^rAde^+$	0	0	0	0	1		279 (69.75)
	0	0	0	1	1		4 (1)
	0	0	1	0	1		8 (2)
	0	0	1	1	1		109 (27.25)
Sm^rGal^+		0	0	0	0	1	336 (84)
		0	0	0	1	1	31 (7.75)
		0	1	0	0	1	1 (0.25)
		0	0	1	0	1	0 (0)
		0	1	0	1	1	1 (0.25)
		0	0	1	1	1	4 (1)
		0	1	1	0	1	3 (0.75)
		0	1	1	1	1	24 (6)

* W1895: $Hfr_1M^-Sm^sThy^+Arg_2^+Ade^+Gal^+$: 1 1 1 1 1 1. JE1040: $F^-M^+Sm^rThy^-Arg_2^-Ade^-Gal^-$: 0 0 0 0 0 0. $Ade^+Sm^rM^+$ recombinants were selected on DM-Glu agar supplemented with 50 μg of arginine, 50 μg of thymine, and 100 μg of streptomycin per ml. Gal^+Sm^r recombinants were selected on EMB-Gal agar supplemented with 50 μg of thymine and 100 μg of streptomycin per ml. Other procedures used were similar to those for Tables 6 and 7. The alleles of markers derived from the Hfr parent are represented by 1, and those derived from the F^- parent, by 0. With the $M^+Sm^rAde^+$ recombinants, 5 of the 400 recombinants were Gal^+. With the Sm^rGal^+, 4 of the 400 recombinants were M^+.

† Numbers in parentheses are percentages of the total. The total number of recombinants examined in both cases was 400.

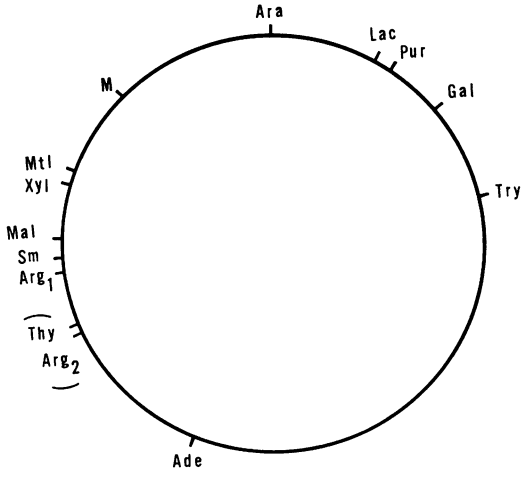

FIG. 1. *Location of the Thy and Arg_2 genes on the chromosome map of Escherichia coli K-12.*

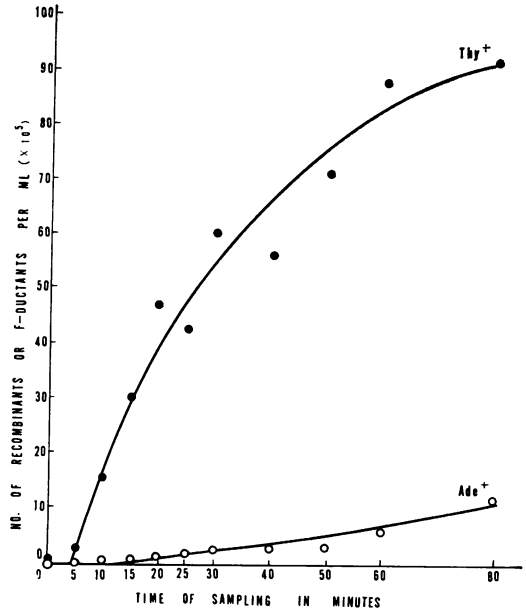

FIG. 2. *Kinetics of transfer of the Thy^+ and Ade^+ genes from W4580 ($F_{15}^+M^-Sm^s$) to JE1040 ($F^-Thy^-Arg_2^-Ade^-Sm^r$). For procedure, see Materials and Methods. Ordinate represents number of Thy^+Sm^r or Ade^+Sm^r cells in the mating mixture; abscissa, the time after the mating was initiated by the mixing of donor and recipient bacteria.*

nevertheless had the F factor, as evidenced by the low frequency transfer of the Thy^+ and Lac^+ genes. Thus, they contain F which is not associated with the Thy^+ gene.

DISCUSSION

From the data presented above the following conclusions are drawn. (i) The gene responsible for thymine requirement or independence is at a locus on the chromosome of *E. coli* K-12, near to the Arg_2 gene and between the *Sm* and *Ade* genes. (ii) A new F′ (F_{15}) carrying the *Thy* locus as well as the Arg_2 locus was discovered. (iii) F_{15} contains the functionally active allele of the mutated loci of all Thy^- mutants of *E. coli* examined as well as Thy^- mutations of *Salmonella typhimurium* and *Serratia marcescens*.

In a preliminary report, Okada observed that some strains of K-12 transfer the *Thy* gene at high frequency independently of the chromosomal linkage, and suggested that the *Thy* gene itself is a new episome (Okada, 1962). This hypothesis is not in agreement with our results and especially

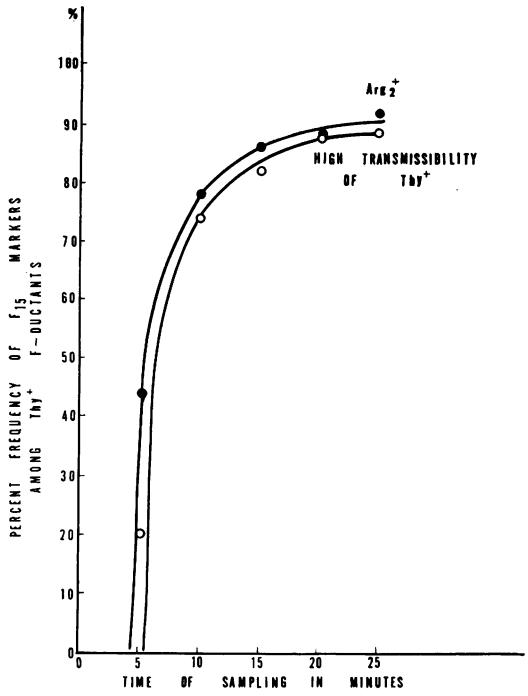

FIG. 3. *Transfer of Arg_2^+ and F from W4580 to JE1040. The ordinate shows the percentage of Thy^+ cells in Fig. 2, carrying Arg_2^+ gene, or carrying F in association with Thy^+; abscissa, as in Fig. 2. All Thy^+ cells tested were Ade^-. Transmissibility of the Thy^+ gene was assayed by replica plating onto EM-Glu plate seeded with JE666 (F^-Thy^-).*

with the observation that F_{15} carries another chromosomal gene, *Arg_2*, with *Thy*. *Thy* was closely linked to *Arg_2* in independent crossing experiments with Hfr and F^-. This indicates that F_{15} originated by incorporation of the chromosomal fragment *Thy-Arg_2* in the F factor. Okada's observations may best be interpreted by assuming a similar F′ carrying the *Thy* gene; he found that the transfer of the *Thy* gene was dependent upon the presence of the F factor. From all these considerations, it seems more reasonable to conclude that the *Thy* gene may sometimes be incorporated into the F factor to form a complex, belonging to the known category of F-primes. Recently, Kitsuji (*personal communication*) independently found that the *Thy* gene is located on the chromosome in the region between *Sm* and *His*.

The enzymatic defect of the thymineless mutants obtained by aminopterine treatment has not been determined, but Barner and Cohen (1959) showed that *E. coli* 15 T^- and B3 were defective in thymidylate synthetase. F_{15} relieves the functional defect of 15 T^-, B3, and aminopterine-mutants, suggesting that aminopterine-mutants may also lack the same enzyme. In view of the clustering of loci for related enzymatic steps in *E. coli*, however, the possibility cannot be excluded that a different but related enzymatic step is involved in aminopterine-mutants.

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