JOURNAL OF BACTERIOLOGY, Oct. 1972, p. 206-211

Vol. 112, No. 1
Printed in U.S.A.

Mapping of the *fabA* Locus for Unsaturated Fatty Acid Biosynthesis in *Escherichia coli*

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Received for publication 10 July 1972

fabA mutants of *Escherichia coli* require an appropriate unsaturated fatty acid for growth. The *fabA* locus has now been mapped at minute 21.5 of the linkage map of *E. coli*. The locus is cotransduced with *pyrD* and *aroA* but not with *pyrC*, *purB*, or *pdxC*. The clockwise order of markers in the region is *pdxC*, *aroA*, *cmlB*, *pyrD*, *fabA*, *pyrC*.

Mutants of *Escherichia coli* defective in unsaturated fatty acid biosynthesis were previously divided into two complementation groups: *fabA* and *fabB* (2). *fabA* mutants are deficient in β-hydroxydecanoylthioester dehydrase (12), the enzyme studied by Bloch and co-workers (4). The biochemical lesion in the *fabB* mutants is unknown. The *fabB* locus maps at minute 44 of the *E. coli* linkage map (3, 9). The *fabA* locus had not been mapped although it was known not to be closely linked to *fabB* (2).

In this paper we report the genetic mapping of the *fabA* locus.

MATERIALS AND METHODS

Bacterial strains. The properties of the *E. coli* strains used in this work are given in Table 1. All strains are derivatives of *E. coli* K-12. Strain UC1 is a str^R F^+ derivative of W3110. W3110 was the gift of M. Meselson. Strain UC1098 was isolated from UC1 by the following procedure. The growth medium used throughout the isolation sequence was medium 56 (6) with 0.4% potassium acetate as sole carbon source. Log-phase cultures of UC1 were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (100 μg/ml) for 20 min at 40 C as described by Adelberg et al. (1). The cultures were grown for three to four generations at 30 C and then plated at 30 C. The resulting colonies were then transferred to two plates, one of which was incubated at 30 C, the other at 40 C. The temperature-sensitive colonies were picked from the 30 C plate and purified by restreaking twice at 30 C. These strains were then tested for the ability to be transduced to wild type by phage P1. Transduction was used as a criterion for temperature sensitivity being caused by single mutational lesions. Of 367 transducible and temperature-sensitive mutants, 2 were found to grow normally at 40 C when supplemented with oleic acid. One of these mutants was UC1098, and the other was UC204, a *fabB* mutant described previously (2).

Media. R broth (RB) contained (in grams/liter): tryptone, 10; glucose, 1; yeast extract, 1; and sodium chloride, 5. The minimal medium used for scoring recombinants was medium E (14) supplemented with 0.4% glucose, 1.5% agar, and thiamine, 1 μg/ml. When required as growth factors, the minimal medium was supplemented with nucleic acid bases at 50 μg/ml, L-amino acids at 25 μg/ml, pantothenic acid at 1 μg/ml, or fatty acids at 100 μg/ml. The fatty acids were solubilized by the addition of 0.1% Brij 58 detergent to the medium. Streptomycin sulfate was added to 200 μg/ml. Nutrient broth (Difco) was prepared as described by the supplier.

Genetic crosses. Conjugational transfer experiments with UC1098 as recipient were carried out in RB medium. The strains were grown at 30 or 37 C to a concentration of 1×10^8 to 2×10^8 cells/ml and then mixed in various donor-recipient ratios and gently shaken for 1 to 2 hr at 33 C. In some experiments the recipients were F^- phenocopies of UC1098 produced by growing the strain overnight at 30 C with vigorous aeration.

The mating shown in Fig. 1 was conducted by mixing Hfr 3000 and L010 str^R cells in a ratio of 1:20 (total of 3×10^8 cells/ml) in oleate-supplemented nutrient broth. The mating mixture was gently shaken at 37 C for 10 min and then diluted 100-fold into medium E (supplemented with 0.2% glycerol and thiamine) and allowed to stand at 37 C. Samples were taken at various time intervals, mixed for 2 min at full speed on a Vortex mixer, and plated on selective media. $fabA^+$ recombinants were scored by the distinctive colony morphology of L010 (11).

Plate crosses were done by spotting exponential cultures of str^S Hfr strains on lawns of str^R recipient cells on streptomycin-containing selective medium at 37 C. The crosses were scored after overnight growth at 37 C.

Phage lysates for transduction were obtained in RB medium by using phage P1 *vir*, hereafter called P1.

TABLE 1. *Bacterial strains*[a]

Strain	Sex	Genotype	Source
UC1	F+	*str*^{R}	See text
UC1098	F+	*fabA2, str*^{R}	From UC1 (see text)
AB2829	F+	*aroA354*	J. Pittard (7) strain
AB478	F-	*aroA2, his-4, proA2, thr-1, mtl-1, xyl-5, galK2, lacY-1,*	J. Pittard (7) strain
1908	F-	*pyrD34, thi-1, his-1, str-118, gal-6, xyl-7, mtl-2, thyA25, glpD3, glpR*^{C}*2*	G. N. Godson
AT3143	F-	*pdxC3, pyrC30, ilv-277, met-65, his-53, purE41, proC24, cyc*^{R}*-1, xyl-14, lacY29, str-97, tsx-66,* λ$^-$, *Su*$^-$	A. L. Taylor strain
KL218	F-	*pyrC30, purE41, thyA25, nalA12, argG34, metB1, his-53, proC24, lac*$^-$, *str-97, tsx-63, mlt-2, xyl-7,* or *14*	K. B. Low strain
AB1325	F-	*proA2, purB15, his-4, thr-1, str-35, lacY1, galK2, mtl-1 xyl-5*	K. B. Low strain
KL185	F-	*pyrD34, thi-1, his-1, str-118, trp-1, gal-6, xyl-7, mtl-2*	K. B. Low strain
L010 *str*^{R}	F+	*fabA1, thi-1, pan-6, str*^{R}	Spontaneous from L010 (2, 12)
YAA1	F-	*fabA2, thi-1, his-1, str-118, trp-1, gal-6, xyl-7, mtl-2*	*pyrD*$^+$, *fabA*$^-$transductant of KL185 by UC1098.
Hfr3000	HfrH	*thi-1, rel-1* λ$^-$	F. Jacob strain
KL99	Hfr	*thi-1, rel-1, lac-42* λ$^-$, λ^R	K. B. Low strain
Hfr6	Hfr	*metB1, mtl-8, mal-20, mut-2,* λ$^-$, λ^R	J. Lederberg strain
C600	F-	*thi-1, thr-1, leu-6, lacY1, tonA21,* λ$^-$, *supE44*	R. Appleyard strain

[a] The genetic nomenclature used is that of Taylor (13). All of the *fabA*$^+$ strains are available from the Coli Genetic Stock Center, Department of Microbiology, Yale University.

The stocks were obtained as previously described (2) except that sodium citrate (2.5 mM) and $MgCl_2$ (10 mM) were added to the lysate to inhibit phage adsorption to bacterial debris and phage inactivation. respectively. The phage stocks were cycled through a given strain at least twice before use and were titered on strain C600. The stocks varied between 10^9 and 10^{10} plaque-forming units per ml. The transducing ability of these lysates was 10^{-5} to 10^{-6} of the plaque-forming ability.

Transductions were begun by mixing 1 ml of a solution 30 mM in $MgCl_2$ and 15 mM in $CaCl_2$ with 1 ml of phage lysate (sterilized with chloroform) and 1 ml of log-phase bacterial culture in RB. The multiplicity of infection was 0.2 to 0.5. After 30 min of adsorption at 30 C the mixture was centrifuged, resuspended in medium E, and plated on selective media.

Unselected markers were scored by transferring recombinant colonies onto the appropriate solid medium with applicator sticks.

Materials. Fatty acids were from the Hormel institute, Austin, Minn. The other biochemicals were from Sigma. Bacterial culture media were from Difco and Bioquest.

RESULTS

Most of the work reported here was performed with strain UC1098, the isolation of which is described above. UC1098 grows normally at 30 C in all media tested but only grows at temperatures above 35 C if supplemented with an appropriate unsaturated fatty acid. The spectrum of unsaturated fatty acids which support growth of UC1098 at 37 C is similar to that of strain L010 (11). Saturated and hydroxy fatty acids cannot support growth at 37 C. If an exponentially growing culture of UC1098 is shifted from 30 to 37 C in the absence of unsaturated fatty acids, growth ceases after one to two generations, and the culture begins to lyse. Previous studies showed UC1098 to be defective in unsaturated fatty acid synthesis in vitro at temperatures above 30 C (2). UC1098 was unable to complement strain L010, the original unsaturated fatty acid auxotroph, either in extract or in genetic complementation tests (2). Several other unsaturated fatty acid auxotrophs were complemented by UC1098 and L010 under the same conditions. Thus, UC1098 and L010 were defective in the same gene, *fabA*. Early observations (Cronan and Silbert, *unpublished data*) indicated that the presence of fatty acid or detergent supplements, or both, in the medium had a deleterious effect on most genetic manipulations. Since UC1098 can be grown at permissive temperatures in the absence of these supplements, we preferred to use this strain in the mapping of *fabA*.

Conjugational mapping of fabA. The ge-

netic mapping of *fabA* was complicated by the erratic behavior of both of the established *fabA* mutants, L010 and UC1098, as recipients in conjugation experiments. These strains and their female derivatives (derived by curing or phenocopy) only occasionally acted as efficient recipients in matings done under a variety of conditions.

However, several successful matings allowed us roughly to position *fabA* on the linkage map. Figure 1 shows the results of an interrupted mating of HfrH with L010 str^R. The mating showed an unexpected delay in transfer of pan^+ but this may be due to the experimental protocol. The important finding was that $fabA^+$ was transferred by HfrH beginning about 20 min after the initiation of pan^+ transfer. These data positioned *fabA* roughly at minute 22 of the standard map. This position was confirmed by the results of plate matings with a UC1098 phenocopy culture used as recipient. These experiments indicated that $fabA^+$ str^R recombinants were formed at a low efficiency with Hfr B7 and Hfr6, but no recombinants were formed with HfrC or Hfr KL99. Considering the origins of transfer of these Hfr strains (Fig. 2), the conjugation data suggested that *fabA* was located close to the origin of transfer of Hfr KL99.

Transductional mapping of fabA. The results of the conjugation experiments led us to test for cotransduction of *fabA* with various of the loci in the region of the KL99 origin. Phage P1 stocks grown on strains carrying mutations in the *pyrD*, *purB*, *pyrC*, *gal*, and *trp* loci were used to transduce UC1098 to $fabA^+$. Cotransduction for *pyrD* and *fabA* was observed in both of the *fabA* strains tested, UC1098 and L010. About 45% of the $fabA^+$ recombinants were also $pyrD^-$ (Table 2, crosses 1 and 2). No cotransduction was observed for the other loci, including those mapping close to *pyrD* (see Table 2, crosses 3 and 4). When P1 stocks grown on UC1098 were used to transduce $pyrD^-$ strains to $pyrD^+$, about 56% of the $pyrD^+$ recombinants were also $fabA^-$ (Table 2, crosses 5 and 6). Thus, *pyrD* and *fabA* are quite close to each other.

Other markers which are of interest because they map in this area are *aroA*, *cmlB*, and *pdxC*. *aroA* and *cmlB* are both cotransducible with *pyrD* (8) and *pdxC* is cotransduced with *aroA* at high frequency (13). The order of *pdxC* and *aroA* with respect to the rest of the linkage map was not established (13). We have examined the cotransducibility of *fabA* with *aroA* and with *pdxC* (*cmlB* is difficult to score [8], and hence we did not examine this marker) and have reexamined the relationship of the *aroA*, *pdxC*, and *pyrD* loci. We found that *fabA* cotransduced with *aroA* (Table 2, crosses

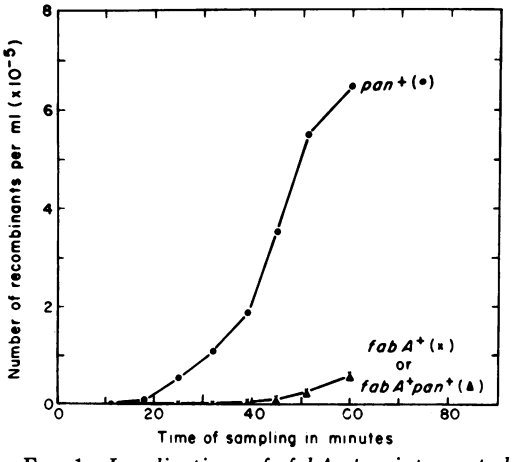

FIG. 1. *Localization of fabA by interrupted mating. Cultures of Hfr3000 and L010 str^R were mixed and allowed to mate for various time intervals before plating. See text for details.*

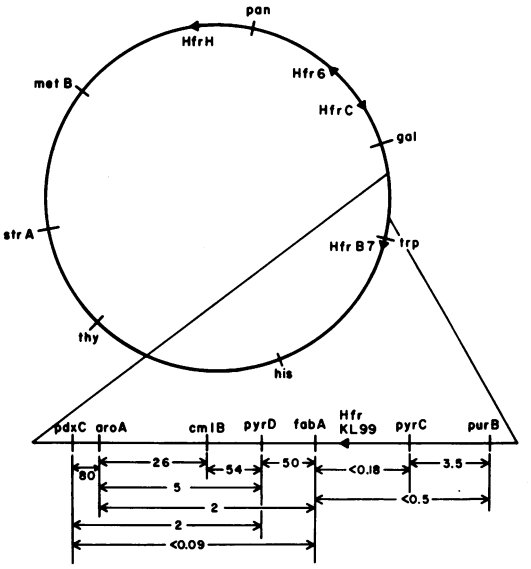

FIG. 2. *Location of fabA on the genetic map of E. coli. The circular map at the top is adapted from that of Taylor (13). The origins and direction of transfer of the Hfr strains used are also given. The figures at the bottom are P1 cotransduction percentages averaged from the data of Table 2, except the aroA-cmlB, cmlB-pyrD, and pyrC-purB frequencies. The first two frequencies are from the work of Reeve and Doherty (8), and the third is from Signer et al. (10). No attempt has been made to draw the enlarged portion of the figure to scale.*

TABLE 2. *Transductional mapping of the fabA locus*

Cross no.	Bacterial strains and relevant markers		Marker selected	Colonies with donor marker[a]/ total colonies scored	Totals	Cotransduction frequency (%)
	Donor	Recipient				
1	1908*pyrD*$^-$	UC1098*fabA*$^-$	*fabA*$^+$[b]	68/148, 79/181	147/329	44.7
2	1908*pyrD*$^-$	L010*fabA*$^-$	*fabA*$^+$	19/58, 53/101, 35/85, 10/24, 17/47, 71/132	205/447	46.0
3	AB1325*purB*$^-$	UC1098*fabA*$^-$	*fabA*$^+$	0/184	0/184	<0.54
4	KL218*pyrC*$^-$	UC1098*fabA*$^-$	*fabA*$^+$	0/217, 0/112, 0/225[c]	0/554	<0.18
5	UC1098*fabA*$^-$	1908*pyrD*$^-$	*pyrD*$^+$	135/241, 175/320	310/561[d]	55.3
6	UC1098*fabA*$^-$	KL185*pyrD*$^-$	*pyrD*$^+$	89/170, 143/233[e]	234/403	58.0
7	UC1098*fabA*$^-$	AB2829*aroA*$^-$	*aroA*$^+$	3/187, 10/586, 10/246	23/1,019	2.3
8	AB2829*aroA*$^-$	UC1098*fabA*$^-$	*fabA*$^+$	3/324	3/324	0.93
9	UC1098*fabA*$^-$	AB478*aroA*$^-$	*aroA*$^+$	9/242	9/242	3.7
10	AB478*aroA*$^-$	UC1098*fabA*$^-$	*fabA*$^+$	5/227	5/227	2.2
11	UC1098*fabA*$^-$	AT3143*pdxC*$^-$	*pdxC*$^+$	0/231, 0/357, 0/117	0/705	<0.14
12	AT3143*pdxC*$^-$	UC1098*fabA*$^-$	*fabA*$^+$	0/225, 0/176	0/401	<0.25
13	1908*pyrD*$^-$	AB2829*aroA*$^-$	*aroA*$^+$	8/225, 15/242	23/467	4.9
14	AB2829*aroA*$^-$	1908*pyrD*$^-$	*pyrD*$^+$	7/160	7/160	4.4
15	AT3143*pdxC*$^-$	1908*pyrD*$^-$	*pyrD*$^+$	2/47, 0/41	2/88	2.3
16	AB2829*aroA*$^-$	AT3143*pdxC*$^-$	*pdxC*$^+$	190/225, 231/242	421/467	90.1
17	AT3143*pdxC*$^-$	AB2829*aroA*$^-$	*aroA*$^+$	47/68	47/68	69.2

[a] Each fraction in this column denotes an experiment done with a different recipient culture and in most cases with different phage stocks. However, a given phage stock was often used as the transductional donor (to wild type) for several different markers. For example, the same two phage stocks were used in crosses 12, 15, and 17, and only three phage stocks were used in crosses 5, 6, 7, 9, and 11.

[b] *fabA*$^+$ was scored by growth of 40 C on media without fatty acid supplementation.

[c] The *pyrC*$^-$ marker of this strain was actually the *pyrC30* marker of AT3143, a parent of KL218.

[d] These and all subsequent *fabA*$^-$ recombinants grow normally at 40 C on media supplemented with oleic acid.

[e] The *pyrD*$^-$ marker of this strain was actually the *pyrD34* marker of KL188, a spontaneous *thyA25* derivative of KL185.

7–10) but not with *pdxC* (Table 2, crosses 11 and 12). The cotransduction frequency for *fabA* and *aroA* (Table 2, crosses 7–10) was about half the cotransduction frequency observed for *pyrD* and *aroA* (Table 2, crosses 13 and 14). Our data agree well with those of Reeve and Doherty (8) and of Taylor (13) for the frequencies of cotransduction of *aroA* with the *pyrD* (Table 2, crosses 13 and 14) and the *pdxC* (Table 2, crosses 16 and 17) loci, respectively. Furthermore, the *pdxC* locus was cotransduced with *pyrD* (Table 2, cross 15) at a frequency half that of the cotransduction frequency of *aroA* with *pyrD* (Table 2, crosses 13 and 14).

Our observations give rise to the following conclusions: (i) *pdxC* and *fabA* are located at opposite ends of this segment of the genetic map; (ii) *pyrD* is closer to *aroA* and *pdxC* than is *fabA*; and (iii) *aroA* is closer to *fabA* and *pyrD* than is *pdxC*. The only map order consistent with these conclusions is *pdxC-aroA-pyrD-fabA*. Although this order is based solely on cotransduction frequencies, it seems reliable because reciprocal transductions give the same map order. The complete transduction data are given in Table 2 and are illustrated in Fig. 2. All of the *fabA*$^-$ recombinants formed in the crosses of Table 2 were able to grow at 40 C only when supplemented with oleic acid.

Order of fabA and the KL99 origin. The origin of transfer of Hfr KL99 is between *pyrD* and *pyrC* (B. Low, *personal communication*) and hence is very close to the *fabA* locus. Therefore, we have ordered *fabA* with respect to the KL99 origin. KL99 began to transfer the *pyrC*$^+$ locus to KL218 during the first 10 min of mating at 33 C, but these conditions gave no transfer either of *fabA*$^+$ to strain YAA1 or of *pyrD*$^+$ to strain 1908. Although *fabA*$^+$ and *pyrD*$^+$ recombinants were formed when mating was allowed to continue for 4 hr at 33 C, no such recombinants were formed when mating was permitted for only 1 hr. Therefore it seemed that *pyrC* was an early marker for KL99 but that both *fabA* and *pyrD* were very late markers. Low recombination frequencies are a property of markers very near the origin of transfer (5) as well as of those markers transferred late in mating. However, the very

TABLE 3. *Order of fabA and the KL99 origin*

Parents		trp^+ str^R Recombinants formed[a]	Total scored	Unselected markers	
Male	Female			*fabA*$^+$	*pyrD*$^+$
KL99	YAA1	8.7×10^5	255	0	
Hfr6	YAA1	2.7×10^5	320	192	
KL99	KL185	8.5×10^5	251		0
Hfr6	KL185	2.1×10^5	219		169

[a] A 1-ml mating mixture of 10^8 cells of a female strain and 10^7 cells of a male strain were shaken slowly at 33 C for 90 min. The cultures were then diluted and plated on selective media at 30 C. The recombinants are expressed per milliliter of mating mixture.

early markers still recombine at a frequency of at least 5 to 10% of that given by proximal markers (5; B. Low, *personal communication*). We therefore performed a more quantitative experiment ordering *fabA* and the KL99 origin. When KL99 was mated with YAA1, less than 0.4% of trp^+ str^R recombinants, formed were also *fabA*$^+$ (Table 3). However, when Hfr6 donated the trp^+ gene, 60% of the recombinants were also *fabA*$^+$. Experiments with the *pyrD* locus of strain KL185 gave similar results. Since none of the trp^+ recombinants formed by transfer from KL99 also obtain *fabA*$^+$ or *pyrD*$^+$, the *fabA* and *pyrD* loci are not transferred by KL99 early in conjugation. Therefore the F factor of KL99 must be inserted between *fabA* and *pyrC* on the side of *fabA* distal to *pyrD* (Fig. 2).

DISCUSSION

The knowledge of the map locations of both *fabA* and *fabB* (3, 9) should greatly facilitate screening for any additional *fab* genes which may exist. F′ episomes are available which contain portions of the genome which should include either *fabA* or *fabB*. Thus, a collection of *fab* mutants could easily be sorted by complementation with such episomes. Sorting could also be done by conjugation or by cotransduction. Any of these methods would be much less cumbersome than the previous methods used to sort *fab* mutants (complementation in extracts or abortive transduction [2]).

The mapping of *fabA* also facilitates the construction of *fabA* mutants of varying genetic backgrounds. One such mutant would possess lesions in both the *fabA* and *fabB* genes. Such double mutants should provide a means for performing experiments which are now thwarted by the reversion of a single mutation to *fab*$^+$. These *fabA-fabB* double mutants may also reveal new aspects of the fatty acid biosynthetic pathway.

The *fabA* lesions in UC1098 and L010 share identical map locations and fail to complement each other in either genetic or extract complementation tests (2). Since L010 is very deficient in the β-hydroxydecanoylthioester dehydrase studied by Bloch and co-workers (4), it seems most likely that *fabA* is the structural gene coding for this enzyme. This has recently been confirmed (Cronan and Gelmann, *manuscript in preparation*) by the finding that the β-hydroxydecanoylthioester dehydrase activity of UC1098 is about 10-fold more thermolabile than the enzyme of its parent, UC1.

ACKNOWLEDGMENTS

We thank Brooks Low and Barbara Bachmann for their valuable advice and for their numerous gifts of strains.

This work was supported by Public Health Service research grants AI-10186 (National Institute of Allergy and Infectious Diseases), GM-16292 (National Institute of General Medical Sciences), and AM-09803 (National Institute of Arthritis and Metabolic Diseases) to J. Cronan, D. Silbert, and D. Wulff, respectively. J. Cronan is supported by Public Health Service Research Career Development Award GM70,411 from the National Institute of General Medical Sciences.

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