JOURNAL OF BACTERIOLOGY, Mar. 1975, p. 1036-1046

Vol. 121, No. 3
Printed in U.S.A.

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

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Received for publication 25 November 1974

fabD mutants of *Escherichia coli* contain a thermolabile malonyl-coenzyme A-acyl carrier protein transacylase which causes defective fatty acid synthesis and temperature-sensitive growth. By conjugation and P1 transduction the *fabD* locus has now been mapped at min 24, between *pyrC* and *purB* and close to *cat*. The order of sites is tentatively given as *pyrC*, *cat*, *fabD*, and *purB*, though the orientation of *cat* and *fabD* could be reversed. The possible relationship of *fabD* with another mutation lying in this region and also affecting acid synthesis is discussed. In the course of these studies we also confirmed the location of the *fabA* gene, determined that *poaA* lies between *fabA* and *pyrC*, and inadvertently found that the *pyr* mutation in strain AT3143 is probably *pyrF* and not *pyrC*.

A number of temperature-sensitive mutants affecting total fatty acid synthesis have been selected in *Escherichia coli* by a [^{3}H]acetate radiation suicide procedure (6). Recently, the malonyl-coenzyme A-acyl carrier protein (ACP) transacylase in two of these strains (LA2-89 and LA2-130) has been shown to be thermolabile (7). By comparing the properties of revertants with that of the original temperature-sensitive mutants, it could be demonstrated that the altered malonyl transacylase activity accounted for the reduced fatty acid synthesis and the cessation of growth at elevated temperatures. Since this enzyme is composed of a single polypeptide chain (8, 15), it could be anticipated that different structural gene mutations for malonyl transacylase would lie in the same locus. In this paper we report the genetic mapping of the two mutations noted above and designate the gene coding for malonyl-coenzyme A-ACP transacylase as the *fabD* locus.

MATERIALS AND METHODS

Materials. Bacterial culture media and Noble agar were purchased from Difco and BBL agar from Bioquest. Oleic acid (>99% pure) was obtained from Hormel Institute, Austin, Minn. Brij 58 (polyoxyethylene cetyl ether) was the gift of Atlas Chemical Co., Wilmington, Del. The dye, 5-bromo-4-chloroindolyl-β-D-galactoside (X-gal), was the product of Cyclo Chemical Co., Los Angeles, Calif. Orthonitrophenyl-β-D-galactoside, δ-aminolevulinic acid, glucose-free lactose, and glucose-free galactose were obtained from Sigma Chemical Co., St. Louis, Mo.

Bacterial strains. The bacterial strains used were all derivatives of *E. coli* K-12. The genotype and source of these strains are given in Table 1. Both *fabD* mutants, strains LA2-89 and LA2-130, were derived from strain AB1623 as described previously (6).

Media. R broth (rB) contained (in g/liter): tryptone, 10; glycerol, 4; yeast extract, 1; and sodium chloride, 5. The minimal medium used for scoring recombinants was medium E (23) supplemented with 0.4% glycerol, 1.5% agar, and 1 μg of thiamine per ml. Overnight inocula of strains with *fab* mutations were grown at permissive temperatures with appropriate growth factors in medium 63 (13) containing 0.4% glycerol, 1 μg of thiamine per ml, and 5 μg of yeast extract per ml. Inocula of other strains were grown in a similar manner or, alternately, on rB medium. For one strain (NG1908) which carried the *glpD* mutation, glucose (0.4%) replaced glycerol as carbon source. Magnesium-calcium buffer used in transductions contained 0.01 M magnesium sulfate and 0.005 M calcium chloride. When required as growth factors, the minimal medium was supplemented with nucleic acid bases at 50 μg/ml, potassium glutamate as 2 mM, other L-amino acids at 25 μg/ml, pantothenic acid at 4 μM, other vitamins (thiamine, *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, pyridoxine) at 1 μg/ml, δ-aminolevulinic acid at 40 μg/ml, and oleic acid at 100 μg/ml. Oleic acid was solubilized by the addition of 400 μg Brij 58 detergent/ml to the medium. When they were provided as carbon source, proline was added at 0.6% and glucose-free galactose or lactose at 0.4%. Streptomycin sulfate was used at 200 μg/ml.

In order to detect inheritance of the *pdxC* marker, the growth requirement for pyridoxine was determined on plates in which washed, BBL, or Noble agar was used. The specific growth requirements of strains carrying mutations in *fabA*, *pyrC*, *purB*, *purE*, *aroA*, and *hemA* loci were as follows: *fabA*, oleic acid; *pyrC*, uracil; *purB* and *purE*, adenine; *aroA*, phenylalanine, tyrosine, tryptophan, *p*-aminobenzoic acid and

TABLE 1. *Bacterial strains used*

Strain	Sex	Genotype	Source
AB470	F⁻	*purB15, thi-1, his-4, proA2, mtl-1, xyl-5, galK2, lacY1, λ⁻, supE44(?)*	A. L. Taylor strain (20) via J. Cronan
AB478	F⁻	*aroA2, thi-1, his-4, proA2, mtl-1, xyl-5, galK2, lacY1*	J. Pittard strain (14) via J. Cronan
AB1325	F⁻	*purB15, thi-1, his-4, proA2, mtl-1, xyl-5, lacY1, galK2, str-35*	A. L. Taylor (20) via J. Cronan
AB2829	F⁺	*aroA354, λ⁻*	J. Pittard strain (14) via J. Cronan
AT3143	F⁻	*pdxC3, pyr[a], ilv-277, met-65, his-53, purE41, proC24, cycA1, xyl-14, lacY29, str-97, tsx-63, λ⁻*	A. L. Taylor strain via J. Cronan
AT3143-T1	F⁻	*purE⁺, pdxC3, pyr[a], ilv-277, met-65, his-53, proC24, cycA1, xyl-14, lacY29, str-97, tsx-63, λ⁻*	Transductant of AT3143 from M226
C600	F⁻	*thi-1, thr-1, leu-6, lacY1, tonA21, λ⁻, supE44*	R. Appleyard strain via J. Cronan
CA198	HfrH	*galU106, rel-1, λ⁻*	S. Brenner strain via CGSC[b]
G19	F⁻	*poaA, purE, gltA, his, lac, strA*	H. Condamine (2)
G19-1	F⁻	*lac⁺, poaA, purE(?), gltA, his, strA*	Recombinant from conjugation of G19 with YAC1
H680	F⁻	*purB51, thi-1, tyrA2, his-68, trp-45, lacY1, gal-6, mtl-2, xyl-7, malA1, str-125, tonA2, tsx-70, λ[R], λ⁻, supE63*	P. G. De Haan strain via CGSC[b]
H680-T1	F⁻	*purB⁺, fabD, thi-1, tyrA2, his-68, lacY1, gal-6, mtl-2, xyl-7, malA1, str-125, tonA2, tsx-70, λ[R], λ⁻, supE63*	Transductant of H680 from LA2-89
Hfr6	Hfr	*metB1, mtl-8, mal-20, mut-2, rel-1, λ⁻, λ[R]*; For origin of transfer, see Low (10)	J. Lederberg strain via J. Cronan
HfrB7	Hfr	*metB1, rel-1, λ⁻, λ[R]*; For origin of transfer, see Low (10)	P. Broda strain via J. Cronan
HfrKL99	Hfr	*thi-1, rel-1, lac-42, λ⁻*; For origin of transfer, see Low (10)	K. B. Low strain via J. Cronan
L010	F⁺	*fabA1, thi-1, pan-6*	Silbert and Vagelos (19)
LA12-G	F⁺	*cat-1*	Loomis and Magasanik (9) via B. Tyler
LA1-6	F⁻	*fab-6, thi-1, gltA, ara-14, lacY1, galK2, xyl-5, mtl-1, tfr-5, tsx-57, str-20, λ* (lysogen)	Harder et al. (6)
LA2-89	F⁻	*fabD1, thi-1, gltA, ara-14, lacY1, galK2, xyl-5, mtl-1, tfr-5, tsx-57, str-20, λ* (lysogen)	Harder et al. (6, 7)
LA2-89e3	F⁻	*gltA⁺, fabD1, thi-1, ara-14, lacY1, galK2, xyl-5, mtl-1, tfr-5, tsx-57, str-20, λ* (lysogen)	Transductant of LA2-89 from NG1908
LA2-89e3L8	F⁻	*lac⁺, fabD1, thi-1(?), ara-14(?), galK2, xyl-5(?), mtl-1(?), tfr-5(?), tsx-57(?), str-20, λ* (lysogen) (?)	Recombinant from conjugation of LA2-89e3 with YAC1
LA2-89e2H2	F⁻	*gal⁺, gltA⁺, fabD1, ara-14, lacY1, xyl-5, mtl-1, tfr-5, tsx-57, str-20, λ* (lysogen)	Transductant of LA2-89 from X8030
LA2-89R11	F⁻	*fabD⁺, thi-1, gltA, ara-14, lacY1, galK2, xyl-5, mtl-1, tfr-5, tsx-57, str-20, λ* (lysogen)	Spontaneous revertant of LA2-89; grows at 38C but not 42C (7)
LA2-130	F⁻	*fabD2, thi-1, gltA, ara-14, lacY1, galK2, xyl-5, mtl-1, tfr-5, tsx-57, str-20, λ* (lysogen)	Harder et al. (6, 7)
LA2-130e1L1	F⁻	*lac⁺, gltA⁺, fabD2, thi-1(?), ara-14(?), galK2, xyl-5(?), mtl-1(?), tfr-5(?), tsx-57(?), str-20, λ* (lysogen) (?)	Recombinant from conjugation of LA2-130 with YAC1
M226	F⁻	*galU106, trp-50, str-150, λ⁻*	J. R. Guest (5)
NG1908	F⁻	*pyrD34, thi-1, his-68, str-118, gal-35, xyl-7, mtl-2, thyA25, glpD3, glpR2, glpK4, phoA8*	G. N. Godson via J. Cronan
S729	F⁻	*hemA, met*	J. Guest (5)
S730	F⁻	*hemA, purB51, thi, trp, his, tyr, pyr, gal, lac, str*	J. Guest (5)
SHSP18	F⁻	*hemA8, metB1, trpA43, lacY1, str-134, malA1(?), λ[R](?), supE44(?)*	A. Sasarman (16)
SHSP18-T1	F⁻	*hemA⁺, purB15, metB1, trpA43, lacY1, str-134, malA1(?)*	Transductant of SHSP18 from AB470
UC1098	F⁺	*fabA2, str-146, λ⁻*	J. Cronan et al. (4)
UC1098-T1	F⁺	*fabA⁺, pyrD, str*	Transductant of UC1098 from NG1908
X7014	F⁻	*pyrC46, purB51, thi-1, lacZ43 or 13, malA1, xyl-7, mtl-2, str-125, λ[R], λ⁻*	J. Beckwith
X7014L	F⁻	*lac⁺, pyrC46, purB51, thi-1(?), malA1(?), xyl-7(?), mtl-2(?), str-125, λ[R](?), λ⁻*	Recombinant from conjugation of X7014 with YAC1
X7014L-T1	F⁻	*purB⁺, fabD1, pyrC46, thi-1(?), malA1(?), xyl-7(?), mtl-2(?), str-125, λ[R](?), λ⁻*	Transductant of X7014L from LA2-89
X7014L-T55	F⁻	*purB⁺, cat-1, pyrC46, thi-1(?), malA1(?), xyl-7(?), mtl-2(?), str-125, λ[R](?), λ⁻*	B. Tyler et al. (22)
X8018	F⁻	*lac015, trp, proC, thi, str, rel-1, λ⁻*	J. Beckwith strain via E. C. C. Lin
X8030	F⁻	*lacY015, trp, proC, thi, str, rel-1, λ⁻*	J. Beckwith strain via E. C. C. Lin
YAC1	HfrC	*fadE, metB, tsx, str, λ⁻*; For origin of transfer, see Low (10)	J. Cronan (3)

[a] Originally assigned as *pyrC* but is probably *pyrF* as shown in the text.

[b] CGSC is abbreviation for *E. coli* Genetic Stock Center, Department of Microbiology, Yale University, New Haven, Conn.

p-hydroxybenzoic acid; *hemA*, δ-aminolevulinic acid. Adequate growth of *hemA* strains required supplementation with δ-aminolevulinic acid even in broth medium.

Solid indicator medium used in screening for catabolite repression of β-galactosidase was prepared as described by Tyler et al. (22). To screen for catabolite repression among *pyrC*$^+$ transductants where the temperature-sensitive *fabD* mutation was an unselected marker, the colonies were grown on the solid indicator medium at 30 C for 36 h rather than at 37 C for 18 h. Colonies with induced levels of β-galactosidase (*cat*$^-$) were detected by their blue color on plates containing the dye known as X-gal (22) or by their deep yellow color following exposure to toluene vapors and then to orthonitrophenyl-β-D-galactoside (9). *cat*$^+$ clones were white in both tests. When the results were equivocal for one of the two procedures, the second method was employed for confirmation.

Genetic crosses. The general map location was obtained by print mapping (11) which relates the proximity of the gene in question to the origin of specific Hfr strains. A series of rapidly growing streptomycin-sensitive Hfr strains was replica plated onto a lawn of the streptomycin-resistant F$^-$ *fabD* mutant which was spread on medium 63 (solid) containing glycerol, thiamine, yeast extract, glutamate and streptomycin. The plates were then incubated at 42 C for 24 h. Clusters of recombinants appeared on the plate in greatest numbers where the F$^-$ strain was exposed to Hfr strains carrying the normal *fabD* allele close to their origin.

In the conjugational transfer experiments, cells from overnight cultures of the F$^-$ recipient (strain LA2-89e3) and the donor Hfr strain were transferred to rB medium and grown at 30 C and 37 C, respectively, to a concentration of 2 to 4 × 10^8 cells/ml. A 0.1 volume of the donor culture was combined with 1 volume of the recipient culture, and the mating mixture was placed at 37 C without shaking. After 5 min, a 0.1 volume of the mixture was transferred with minimal agitation to 1 volume of medium 63 containing 0.1% glycerol and previously equilibrated at 33 C. One-milliliter samples were removed then at 3- or 5-min time intervals, vigorously agitated on a blendor as described earlier (12), diluted appropriately, and plated on selective media containing streptomycin (200 μg/ml) to counter select against the donor.

Phage lysates for transductions were produced by growing phage P1*vir* on appropriate bacterial strains in rB medium. The details of the procedure were as follows. A sample (0.3 ml) of the bacteria in exponential growth (about 1 × 10^8 to 2 × 10^8 cells) and 0.15 ml of one of various dilutions of the phage stock (about 10^6 to 10^8 plaque formers) were added to 3 ml of rB soft (0.45%) agar containing 3.3 mM $CaCl_2$ and incubated at 42 C for 20 min. The absorption mixture was layered onto a warm, freshly poured rB agar plate and incubated at 30 C overnight for bacteria with temperature-sensitive *fab* mutations or otherwise at 37 C for about 6 h. The top agar was scraped off the plates showing confluent lysis (usually about 1 ml per plate) and combined in a centrifuge tube together with 0.5 volumes of rB medium used to rinse the scraped plates. A 0.1 volume of a solution containing 100 mM magnesium sulfate and 50 mM sodium citrate and a 0.001 volume of chloroform were added to the lysate, and the tube was agitated at 30 C for 1 h. The agar and bacterial debris were removed by centrifugation at 10,000 × *g* for 10 min. The supernatant fluid was titered on strain C600 and usually contained between 10^9 and 10^{10} plaque-forming units per ml. The phage stocks were cycled through a given bacterial strain at least twice before use and their transducing abilities ranged from 2 × 10^{-8} to 2 × 10^{-5} of the plaque-forming units.

Most of the transductions were conducted as follows. Cells from overnight cultures were suspended in rB medium at 10^8 cells per ml, grown at permissive temperatures for about two generations (3 × 10^8 to 6 × 10^8 cells per ml), collected by centrifugation, and suspended at the same cell density in magnisium-calcium buffer. (Sometimes the cells were washed once in this buffer to reduce the level of nutrients carried over from rB medium.) A sample (0.1 ml) of the bacterial suspension was combined with 0.1 ml of various dilutions of the appropriate P1*vir* stock to give a multiplicity of infection ranging from 0.02 to 1.6. The mixture was incubated at 37 C for 20 min. After this absorption phase, 3 ml of top (0.75%) agar in minimal medium containing 6.8 mM sodium citrate was added to the absorption tube, the entire contents were plated on selective media, and the plates were incubated at either 42, 30, or 37 C as follows. To minimize reversion in strain LA2-89 during the isolation of *fabD*$^+$ transductants, the selection plates were transferred immediately after the cells were plated to an incubator which was maintained carefully at 42 C. For the optimum selection of *fabD*$^+$ transductants in strain LA2-130, the absorption phase was conducted at 33 C for 60 min, the cells were plated on a minimal medium containing 1 μg of oleic acid/ml, and the plates were incubated initially at 30 C for 30 min and then shifted to 42 C. To obtain *fab*$^+$ transductants from strains carrying the temperature-sensitive *fabA2* mutations, the cells were layered on media in the absence of unsaturated fatty acid supplement, and the plates were incubated at 42 C. Selection plates for transductions involving temperature-sensitive *fabA* or *fabD* as unselected markers were incubated at 30 C. For all other transductions, selections were conducted at 37 C. When strain X7014L-T55 was used as a recipient, the following transduction procedure adapted from Guest (5) was employed. Stationary-phase bacteria from an overnight culture were collected, suspended in magnesium-calcium buffer at about 2 × 10^9 cells per ml, and left at room temperature for 30 min. P1*vir* from the appropriate stock was added to the bacterial suspension to give a multiplicity of infection of 0.4 to 0.7. This mixture was incubated at 33 C for 30 min and centrifuged to separate the cells from the unabsorbed phage. The cell pellet was resuspended in 0.85% sodium chloride and appropriate dilutions were plated on selective media.

All transductions regardless of method used were

controlled by carrying the bacteria and the phage separately through the entire procedure. Growth was absent on the control plates in all but a few experiments where the number of colonies on the bacterial control was less than 5% that obtained in the cross.

Unselected markers were scored by transferring recombinant colonies onto the appropriate solid medium with sterile toothpicks and incubating at the appropriate temperature(s). Donor and recipient bacteria were inoculated on the same plates as controls.

RESULTS

Conjugational mapping of fab-89. Preliminary print mapping of *fab-89* with a series of Hfr strains placed *fab-89* between 10 and 29 min (the origins of entry of strains Hfr6 and HfrB7, respectively) on the chromosome of *E. coli*. The location of the *fab-89* mutation was defined further by an interrupted mating experiment with strain Hfr-KL99, which transfers the chromosome in a clockwise fashion starting at about min 23. *fab*$^+$ recombinants appeared in strain LA2-89 within 7 min after mating commenced with strain HfrKL99 (data not shown). These results taken together with those obtained by print mapping indicated that *fab-89* was between min 23 and 29. Since strain LA2-89 contained *lac* and *gal* mutations as well as *fab-89*, a more detailed mating was performed with strains Hfr6 and HfrB7 in order to relate the position of *fab-89* to the known location of the other two sites. A *gltA* derivative (strain LA2-89e3) was constructed by transduction to eliminate the requirement for glutamate and, hence, to provide a suitable recipient for the selection of *lac*$^+$ or *gal*$^+$ recombinants. The *fab*$^+$ allele is transferred from strain Hfr6 to strain LA2-89 *gltA*$^+$ approximately 7 and 14 min, respectively, after the *gal*$^+$ and *lac*$^+$ alleles (Fig. 1). The low level of *lac*$^+$ recombinants can be attributed to the close proximity of the *lac* genes to the origin of strain Hfr6. In an interrupted mating with strain HfrB7, the order of transfer of markers was reversed as anticipated and, although there was more scatter in the experimental points due to technical difficulties, the time intervals between the appearance of *fab*, *gal*, and *lac* markers was approximately the same as those observed in the cross with strain Hfr6 (data not given). These results placed the site of the *fab-89* mutation at about min 24 on the chromosome.

Transductional mapping of fabD. In order to establish the location of *fab-89*, we tested for co-transduction between the *fab-89* site and various loci in the region from 20 to 27 min (Fig. 2). Phage stocks that were prepared by growing P1*vir* on strains carrying mutations in the *pdxC*,

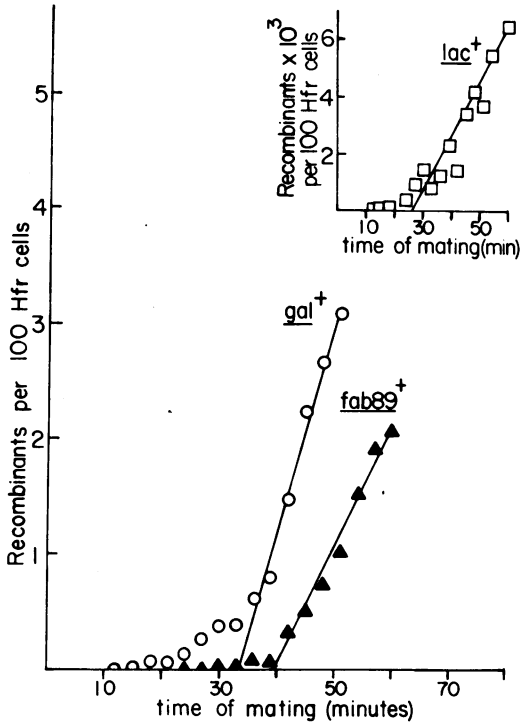


FIG. 1. *Localization of fabD by interrupted mating. Cultures of strains Hfr6 and LA2-89e3 were grown and used in an interrupted mating experiment as described in the text. Note that the emergence of lac*$^+$ *colonies is shown in the insert on a separate scale due to the low level of recombinants.*

aroA, *pyrD*, *fabA*, *poaA*, *pyrC*, *cat*, *purB*, *hemA*, *galU*, and *trp* loci were used to transduce strain LA2-89 to *fab*$^+$ (Table 2, crosses 1 thru 21; marker selected designated *fabD*$^+$). The data demonstrate that *poaA*, *pyrC*, *cat*, *purB*, and *hemA*, markers between min 23 and 26, all co-transduce with *fab-89* (Table 2, crosses 6 through 19). Based on frequencies of co-transduction, *fab-89* is closest to the *cat* locus at min 24.3. In the course of these experiments, we found that the *pyr* mutation in strain AT3143, previously designated as *pyrC*, was not co-transduced with *fab-89* (cross 22). The probable explanation for this unexpected result is given below. When the reciprocal crosses between P1*vir* grown on strain LA2-89 and strains carrying mutations in *pyrC*, *purB*, *hemA*, *galU*, and *trp* were attempted (Table 2, crosses 23 through 31), co-transduction was observed at the anticipated level only with the *purB* site (crosses 26 and 27). Transfer of *fab-89* during selection for *pyrC*$^+$ was rare (compare crosses 23 to 25 with crosses 7 to 9). The recovery of *fab-89* transduc-

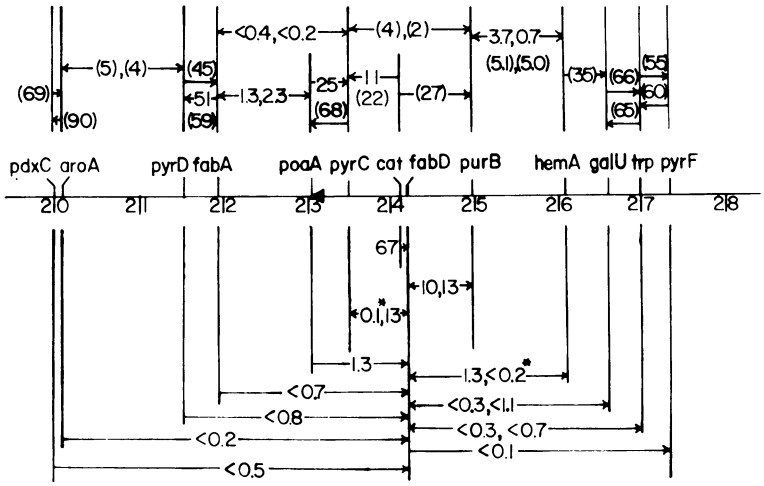


FIG. 2. *Location of fabD on the genetic map of E. coli. The portion of the chromosome containing genetic loci from 20 to 28 min is shown here. It was adapted from the map of Taylor and Trotter (21) differing only with respect to the inclusion of the origin of HfrKL99 (shown by the arrowhead, ◀), placement of poaA to the left rather than to the right of pyrC, and inclusion of the fabD locus. The numbers shown in parentheses above the map are co-transduction frequencies taken from earlier work: pdxC-aroA, aroA-pyrD, and pyrD-fabA from Cronan et al. (4); poaA-pyrC from Condamine (2); pyrC-cat and cat-purB from Tyler et al. (22); pyrC-purB from Signer et al. (17); purB-hemA, hemA-galU, and galU-trp from Guest (5); and trp-pyrF from Signer et al. (17). The values that are not in parentheses are co-transduction percentages averaged from the experiments given in Tables 2, 3, and 4. The head of the arrow points to the marker selected, and where reciprocal crosses were performed, the co-transduction frequency corresponding to each selection is placed nearer to the marker selected. Linkages given for fabD are based on data in Table 2 obtained with the fabD1 mutation. Anomalous results observed when fabD⁻ was an unselected marker (see text) are identified by an asterisk.*

tants was not improved by supplementation of the selection plates with various nutrients that might prevent potentially adverse metabolic effects in cells with a defective malonyl-coenzyme A-ACP transacylase (see Table 2, footnotes *d* through *g* [crosses 23 and 24]).

Strain LA2-130, as noted in the introduction, is another strain containing a different temperature-sensitive mutation in malonyl transacylase (7). Therefore, it was important to determine if this mutation co-transduced with the loci in the 23 to 24 min region in a manner analogous to *fab-89*. In Table 2 (crosses 32–36), it can be seen that this is the case although co-transduction frequencies between *poaA*, *pyrC*, *cat*, or *purB* and *fab-130* in general were higher than those measured between these sites and *fab-89*. In the reciprocal cross between P1*vir* grown on strain LA2-130 and a strain carrying a *pyrC* mutation, co-transduction was suppressed (Table 2, cross 37) just as it was in the analogous cross involving P1*vir* grown on strain LA2-89 (Table 2, cross 24).

Since *fab-89* and *fab-130* are temperature-sensitive mutations affecting malonyl transacylase and both occur at the same position on the chromosome, this locus is the structural gene for this enzyme and will be designated hereafter as *fabD*.

Mapping of markers near the fabD locus. In the course of determining the linkage between *fabD* and various markers in the 23 to 25 min region, we reexamined the co-transduction frequencies of *poaA*, *cat*, and *purB*, with *pyrC*. We have confirmed the observation made by Condamine (2) that *poaA* is close to *pyrC* (68%) although the linkage was somewhat lower (25.4%) in our experiments (Table 3, cross 1). Since *poaA* is co-transduced with *pyrC* at a much higher frequency than with *fabD* (1.3 and 1.9% as measured in crosses 6 and 32, Table 2) *poaA* would appear to lie on the side of *pyrC* away from *fabD*. This conclusion is further supported by experiments which show that strain HfrKL99 transfers *pyrC* but not *poaA* as an early marker. Strains carrying *poaA* and *his* (strain G19), *pyrC* (strain X7014), and *trp* (strain X8030) were crossed with strain Hfr6 and with strain HfrKL99 for 60 min, the conjugations were then interrupted by blending, and the cells were plated on appropriate selection media with streptomycin as a counterselective agent. When strain Hfr6 was the donor, recombinants for *poaA*, *pyrC*, *trp*, and *his* were

TABLE 2. *Transductional mapping of the fabD locus*[a]

Cross no.	Bacterial strains and relevant markers		Marker selected	Colonies with donor marker[b]/ total colonies scored	Co-transduction frequency %
	Donor	Recipient			
1	AT3143 *pdxC*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	0/211	<0.47
2	AB2829 *aroA*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	0/182, 0/306	<0.20
3	AB478 *aroA*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	0/36	<2.8
4	NG1908 *pyrD34*	LA2-89 *fabD*$^-$	*fabD*$^+$	0/25, 0/99	<0.81
5	L010 *fabA*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	0/136	<0.74
6	G19 *poaA*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	0/34, 3/204	1.3
7	X7014 *pyrC*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	27/208	13.0
8	X7014L-T55 *pyrC*$^-$	LA2-89e3 *fabD*$^-$	*fabD*$^+$	11/102	11.0
9	X7014L-T55 *pyrC*$^-$	LA2-89e3L8 *fabD*$^-$	*fabD*$^+$	9/58	15.5
10	LA12-G *cat-1*	LA2-89e3L8 *fabD*$^-$	*fabD*$^+$	12/18, 38/57	66.7
11	X7014L-T55 *cat-1*	LA2-89e3L8 *fabD*$^-$	*fabD*$^+$	39/58	67.4
12	AB1325 *purB15*	LA2-89 *fabD*$^-$	*fabD*$^+$	10/131, 48/345	12.2
13	AB470 *purB15*	LA2-89 *fabD*$^-$	*fabD*$^+$	39/317	12.3
14	H680 *purB51*	LA2-89 *fabD*$^-$	*fabD*$^+$	12/177	7.3
15	S730 *purB51*	LA2-89 *fabD*$^-$	*fabD*$^+$	35/416, 7/44	9.1
16	X7014 *purB*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	13/208	6.2
17	SHSP18 *hemA8*	LA2-89 *fabD*$^-$	*fabD*$^+$	6/161, 0/77, 6/110	3.4
18	S729 *hemA*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	1/415, 9/686	0.91
19	S730 *hemA*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	3/416	0.72
20	M226 *galU*$^-$	LA2-89e2H3 *fabD*$^-$	*fabD*$^+$	0/338	<0.30
21	X8030 *trp*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	0/78, 0/296	<0.27
22	AT3143 *pyr*$^-$	LA2-89 *fabD*$^-$	*fabD*$^+$	0/211, 0/486	<0.14
23	LA2-89 *fabD*$^-$	X7014 *pyrC*$^-$	*pyrC*$^+$	0/46, 0/116, 0/416[d]	<0.17
24	LA2-89 *fabD*$^-$	X7014L-T55 *pyrC*$^-$[c]	*pyrC*$^+$	0/19, 0/21, 0/244[e], 0/102[e] 1/92[e], 1/189[f], 0/204[g]	0.23
25	H680-T1 *fabD*$^-$	X7014L-T55 *pyrC*$^-$	*pyrC*$^+$[c]	0/201	<0.50
26	LA2-89 *fabD*$^-$	H680 *purB51*	*purB*$^+$	4/20, 8/82	11.8
27	LA2-89 *fabD*$^-$	S730 *purB51*	*purB*$^+$	0/16, 1/13, 14/80	13.8
28	LA2-89 *fabD*$^-$	SHSP18 *hemA8*	*hemA*$^+$	0/129, 0/361	<0.20
29	LA2-89 *fabD*$^-$	CA198 *galU*$^-$	*galU*$^+$	0/46, 0/43	<1.1
30	LA2-89 *fabD*$^-$	X8030 *trp*$^-$	*trp*$^+$	0/35	<2.9
31	LA2-89 *fabD*$^-$	X8018 *trp*$^-$	*trp*$^+$	0/113	<0.88
32	G19 *poaA*$^-$	LA2-130e1L1 *fabD*$^-$	*fabD*$^+$	1/16, 1/87	1.9
33	X7014L-T55 *pyrC*$^-$	LA2-130 *fabD*$^-$	*fabD*$^+$	18/63	28.6
34	X7014L-T55 *pyrC*$^-$	LA1-130e1L1 *fabD*$^-$	*fabD*$^+$	16/52	30.8
35	X7014L-T55 *cat-1*	LA2-130e1L1 *fabD*$^-$	*fabD*$^+$	45/52	86.5
36	AB470 *purB15*	LA2-130 *fabD*$^-$	*fabD*$^+$	17/98, 51/302	17.0
37	LA2-130 *fabD*$^-$	X7014L-T55 *pyrC*$^-$	*pyrC*$^+$[c]	0/102	<0.98

[a] The selection media and transduction procedures are described in the text. Note that the conditions of transduction were modified when strains X7014L-T55 and LA2-130 or its derivatives were recipients.

[b] Each fraction represents the results of a separate experiment with a different recipient culture and usually, though not always, with the same phage P1*vir* stock.

[c] Selection for *pyrC*$^+$ was done with a mixture of all the amino acids (0.15% Casamino Acids or 0.05% of a synthetic mixture) since strain X7014L-T55 grew poorly on glycerol with pyrimidines as the only supplement. See reference 22 for growth characteristics of this strain and others carrying the *cat-1* mutation.

[d] This transduction was conducted under the conditions described in the text for strain X7014L-T55.

[e] The selection plate contained Brij 58 (200 μg/ml) and potassium oleate (50 μg/ml).

[f] The selection plate contained Brij 58 (200 μg/ml), potassium oleate (50 μg/ml), and calcium pantothenate (4 μM).

[g] The selection plate contained Brij 58 (200 μg/ml), potassium oleate (50 μg/ml), and yeast extract (5 μg/ml).

obtained with frequencies per 100 Hfrs of 1.0, 1.3, 4.5, and 0.04, respectively; when strain HfrKL99 was the donor, recombinants for these same markers were recovered with frequencies of <0.003, 0.16, 16.7, and 0.25. None of 74 *his*$^+$ recombinants of strain G19 crossed with strain KL99 received the wild-type allele for *poaA*, whereas three out of nine *his*$^+$ recombinants obtained in the cross of strain G19 with strain Hfr6 were also *poaA*$^+$. These results indicate that the origin of strain KL99 is probably between *poaA* and *pyrC* (see Fig. 2). Also, they

TABLE 3. *Transductional mapping of markers near the fabD locus*[a]

Cross no.	Bacterial strains and relevant markers		Marker selected	Colonies with donor marker[b]/ total colonies scored	Co-transduction frequency
	Donor	Recipient			
1	G19 *poaA*$^-$	X7014L-T55 *pyrC*$^-$	*pyrC*$^+$	78/307	25.4
2	LA12-G *cat-1*	X7014L *pyrC*$^-$	*pyrC*$^+$	33/210	15.7
3	G19 *cat*$^+$	X7014L-T55 *pyrC*$^-$	*pyrC*$^{+c}$	21/307	6.8
4	M226 *cat*$^+$	X7014L-T55 *pyrC*$^-$	*pyrC*$^{+c}$	2/20, 14/144	9.8
5	LA1-6 *cat*$^+$	X7014L-T55 *pyrC*$^-$	*pyrC*$^{+c}$	12/73, 17/133	14.1
6	LA2-89 *cat*$^+$	X7014L-T55 *pyrC*$^-$	*pyrC*$^{+c}$	0/19, 2/21, 5/244[e], 0/102[e], 2/92[e], 3/189[f], 1/204[g]	1.5
7	H680-T1 *cat*$^+$	X7014L-T55 *pyrC*$^-$	*pyrC*$^{+c}$	4/201	2.0
8	LA2-89R11 *cat*$^+$	X7014L-T55 *pyrC*$^-$	*pyrC*$^{+c}$	4/204	2.0
9	LA2-130 *cat*$^+$	X7014L-T55 *pyrC*$^-$	*pyrC*$^{+c}$	1/102	1.0
10	LA12-G *purB*$^+$	X7014L *pyrC*$^-$	*pyrC*$^+$	0/210	<0.48
11	LA2-89 *purB*$^+$	X7014 *pyrC*$^-$	*pyrC*$^+$	0/46, 0/116, 0/416[d]	<0.17
12	LA2-89 *hemA*$^+$	S730 *purB51*	*purB*$^+$	1/16, 2/13, 1/80	3.7
13	AB470 *purB15*	S729 *hemA*$^-$	*hemA*$^+$	4/520	0.77
14	AB470 *purB15*	SHSP18 *hemA8*	*hemA*$^+$	1/231, 4/520	0.67
15	AB470 *purB15*	AT3143-T1 *pyr*$^-$	*pyr*$^+$	0/520	<0.19
16	AT3143 *pyr*$^-$	SHSP18-T1 *purB15*	*purB*$^+$	0/52	<1.9
17	AT3143 *pyr*$^-$	SHSP18 *hemA8*	*hemA*$^+$	4/520	0.77
18	AT3143 *pyr*$^-$	S729 *hemA*$^-$	*hemA*$^+$	4/598	0.67
19	SHSP18 *hemA8*	AT3143 *pyr*$^-$	*pyr*$^+$	1/191	0.52
20	S729 *hemA*$^-$	AT3143 *pyr*$^-$	*pyr*$^+$	23/519	4.4
21	X8030 *trp*	AT3143 *pyr*$^-$	*pyr*$^+$	99/207	47.8
22	AT3143 *pyr*$^-$	X8030 *trp*$^-$	*trp*$^+$	101/520	19.4

[a] The selection media and transduction procedures are described in the text. Note that the conditions of transduction were modified when strains X7014L-T55 and LA2-130 or its derivatives were recipients.

[b] Each fraction represents the results of a separate experiment with a different recipient culture and usually, though not always with the same phage P1*vir* stock.

[c] Selection for *pyrC*$^+$ was done with a mixture of all the amino acids (0.15% Casamino Acids or 0.05% of a synthetic mixture) since strain X7014L-T55 grew poorly on glycerol with pyrimidines as the only supplement. See reference 22 for growth characteristics of this strain and others carrying the *cat-1* mutation.

[d] This transduction was conducted under the conditions described in the text for strain X7014L-T55.

[e] The selection plate contained Brij 58 (200 μg/ml) and potassium oleate (50 μg/ml).

[f] The selection plate contained Brij 58 (200 μg/ml), potassium oleate (50 μg/ml), and calcium pantothenate (4 μM).

[g] The selection plate contained Brij 58 (200 μg/ml), potassium oleate (50 μg/ml), and yeast extract (5 μg/ml).

are consistent with the assignment of *poaA* to the *fabA* side of *pyrC*. This order is confirmed below (Table 4) with the demonstration that *poaA* but not *pyrC* is co-transduced with *fabA*, which lies at min 22.

The co-transduction between *cat* and *pyrC* in these studies (ranging from 6.8 to 15.7% in crosses 2 through 5, Table 3) was slightly lower than that reported previously (22%; [22]). Moreover, the linkage between these two sites was markedly reduced in all crosses involving *fabD*$^-$ strains (1 to 2%, crosses 6 through 9, Table 3). The normal linkage was observed in a transduction involving P1*vir* grown on strain LA1-6 (cross 5, Table 3), a strain that was derived like strains LA2-89 and LA2-130 from strain AB1623 (6) but contains a different temperature-sensitive *fab* mutation that lies at min 44 (18). However, the co-transduction frequency between *cat* and *pyrC* was not restored to normal by the addition of various nutrients to the selection plates (see Table 3, footnotes *e* thru *g* [cross 6]) or by genetic manipulations such as transferring *fabD1* into another genetic background (strain H680-T1 used in cross 7, Table 3), selection of a partial revertant of *fabD1* (strain LA2-89 R11 used in cross 8, Table 3), or use of another *fabD* mutation (strain LA-130 used in cross 9, Table 3). Furthermore, in contrast to earlier reports (17, 22), we were unable to detect co-transduction between *purB* and *pyrC* (crosses 10 and 11, Table 3) by using P1*vir* grown on strains containing *cat* (cross 10, Table 3) or *fabD* (cross 11, Table 3) mutations.

purB and *hemA* were shown to co-transduce with a frequency ranging from 0.67 to 3.7 (crosses 12 to 14, Table 3) with the lower level seen in crosses involving transfer of the *purB*$^-$

TABLE 4. *Transductional mapping of fabA locus*[a]

Cross no.	Bacterial strains and relevant Markers		Marker selected	Colonies with donor marker[b]/total colonies scored	Co-transduction frequency
	Donor	Recipient			
1	L010 *fabA1*	NG1908 *pyrD34*	*pyrD*$^+$	43/59	72.9
2	L010 *fabA1*	UC1098-T1 *pyrD34*	*pyrD*$^+$	33/93	35.0
3	UC1098 *fabA2*	NG1908 *pyrD34*	*pyrD*$^+$	17/31	54.8
4	L010 *fabA1*	G19 *poaA*$^-$	*poaA*$^+$	5/211	2.3
5	G19 *poaA*$^-$	UC1098 *fabA2*	*fabA*$^+$[c]	4/306	1.3
6	L010 *fabA1*	X7014 *pyrC*$^-$	*pyrC*$^+$	0/312	<0.32
7	UC1098 *fabA2*	X7014 *pyrC*$^-$	*pyrC*$^+$	0/86, 0/208	<0.34
8	X7014 *pyrC*$^-$	UC1098 *fabA2*	*fabA*$^+$[c]	0/59, 0/177	<0.42

[a] The selection media and transduction procedures are described in the text. Note that the conditions of transduction were modified when strains X7014L-T55 and LA2-130 or its derivatives were recipients.

[b] Each fraction represents the results of a separate experiment with a different recipient culture and usually, though not always, with the same phage P1*vir* stock.

[c] Strain UC1098 grows normally at 30 C without fatty acids but requires unsaturated fatty acid supplement at higher growth temperatures. *fabA* selection was for growth at 40 C without fatty acid supplementation. Brij 58 and potassium oleate were omitted from the absorption mixture also because of their deleterious affect on genetic manipulations as noted earlier (4; J. H. F. F. Broekman, Ph.D. thesis, 1973).

allele (also observed by J. Guest, personal communication).

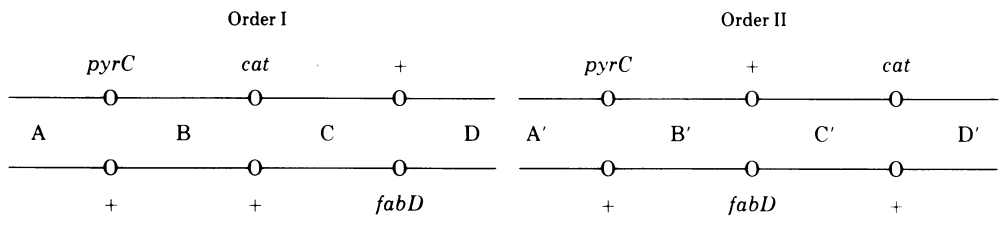
During our investigations on the linkage of *pyrC* and *fabD*, we found that the *pyrC* mutation in strain X7014, but not that in strain AT3143, was co-transduced with *fabD*. These results suggested that the assignment of the *pyr* mutation in strain AT3143 as a *pyrC* defect was incorrect. This hypothesis has been confirmed by the demonstration that this *pyr* mutation is co-transduced with markers at min 26 and 27 (crosses 17 thru 22, Table 3) but not with markers at min 24 and 25 (cross 22, Table 2 and crosses 15 and 16, Table 3). Since this *pyr* mutation is near *trp* (crosses 21 and 22, Table 3), it seems likely that strain AT3143 contains a *pyrF* mutation which lies to the 28-min side of *trp*.

Transductional mapping of the fabA locus. In an earlier study (4), *fabA* was mapped by transduction with respect to markers on its 21-min side, *pyrD* being the most closely linked of the known genes in this region (crosses 1 through 3, Table 3 and reference 3). No linkage was detected by transduction between *fabA* and markers on its 23-min side. Strain AT3143 was used as a source of a *pyrC* mutation in that study. It became necessary to reexamine the linkage of *poaA* and *pyrC* with *fabA* when we determined the orientation of *poaA* with respect to *pyrC* (see above) and recognized the incorrect assignment of the *pyr* locus in strain AT3143 (see above). Crosses 4 through 8 (Table 4) show that *fabA* is co-transduced at a low frequency with *poaA* but not with *pyrC*. These results confirm that *poaA* is situated between *fabA* and *pyrC* and that the distance between *fabA* and *pyrC* is close to or greater than 1.8 min, the maximum interval over which linkage can be detected by P1 transduction.

Three-factor crosses between pyrC, cat, and fabD mediated by P1 transduction. Several difficulties were encountered that interfered with both the construction of suitable strains and the selections necessary for three-factor crosses involving either *pyrC*, *cat*, and *fabD* or *purB*, *cat*, and *fabD*. As noted above, co-transduction of *fabD*$^-$ with *pyrC*$^+$ was very low (Table 2, crosses 23 to 25, 38) relative to that of *pyrC*$^-$ with *fabD*$^+$ (Table 2, crosses 7 to 9, 34, 35). Furthermore, co-transduction of *cat* with *pyrC* was also suppressed when the donor carried the *fabD*$^-$ rather than the *fabD*$^+$ allele (compare crosses 6 to 9 with 2 to 5 in Table 3). We could not circumvent these obstacles by utilizing the co-transduction of *fabD*$^-$ with *purB*$^+$. Despite numerous attempts involving various modifications in the transduction procedure, very few transductants were obtained when *purB*$^+$ recombinants were selected in strains X7014 (*pyrC*$^-$ *purB*$^-$) or its *lac*$^+$ derivative by using P1*vir* grown on strain LA2-89 (*fabD1*$^-$) as donor.

The results of two transductional crosses that were conducted are shown in Table 5. For the second cross, we also attempted to correct the results to reflect normal co-transduction frequencies for *pyrC* with *cat* (14%, Table 3, cross 5) and with *fabD* (13%, Table 2, average of crosses 7 to 9). It is assumed, in doing so, that the anomalous results noted above arise from an interference with recombination in the region of

TABLE 5. *Three-factor crosses between pyrC, cat, and fabD mediated by P1 transduction*



Selected marker (with no. scored)	Resulting characteristics			No. of transductants (%) of total)	Corrected frequency[c] (% of total)	Minimal no. of crossovers required[d]	
	fabD	*cat*	*pyrC*			Order I	Order II
fabD (58)[a]	+	+	+	27.5	NA[e]	2 (C,D)	2 (B',C')
	+	−	+	56.8	NA	2 (B,D)	2 (B',D')
	+	+	−	5.2	NA	4 (A–D)	2 (A',C')
	+	−	−	10.3	NA	2 (A,D)	2 (A',D')
pyrC (871)[b]	+	+	+	1.5	1.3	2 (A,C)	4 (A'–D')
	+	−	+	98.3	85.8	2 (A,B)	2 (A',B')
	−	+	+	0.0	12.7	2 (A,D)	2 (A',D')
	−	−	+	0.2	0.2	4 (A–D)	2 (A',C')

[a] Strain LA2-89e3L8 (*pyrC*$^+$ *cat*$^+$ *fabD1*) was transduced by phage P1*vir* grown on strain X7014L-T55 (*pyrC*$^-$ *cat*$^-$ *fabD*$^+$) as described in the text.

[b] Same as footnote *a* except that strain X7014L-T55 was the recipient and strain LA2-89e3L8, the donor.

[c] Co-transduction of *pyrC*$^-$ with *fabD*$^+$ in the same strains or in strains closely related to those used here averaged 13% (Table 2, crosses 7–9); similarly, co-transduction of *cat*$^+$ with *pyrC*$^+$ was 14% (Table 3, cross 5). In correcting the frequencies obtained in the second cross shown in this table, we have assumed that failure to survive of one or more recombinant classes was not a factor but that the lack (0/871) of *pyrC*$^+$ *cat*$^+$ *fabD*$^-$ recombinants was due to genetic interference with this particular recombinational event. Hence, we have computed that the cross should have yielded an additional 127 *pyrC*$^+$ transductants of the *pyrC*$^+$ *cat*$^+$ *fabD*$^-$ class in order for the results to be consistent with the normal co-transduction frequencies of *fabD* and *cat* with *pyrC*.

[d] The letters in parentheses refer to the regions in which the required crossovers occur (see the two schemes shown at the top of this table).

[e] NA, Not applicable. The co-transduction of *fabD* with *pyrC* and with *cat* in this type of cross are taken as the correct frequencies and the results in this experiment are consistent with the others reported in Table 2 (crosses 7, 8, 10).

fabD$^-$ rather than from a failure to survive of the recombinants carrying this region. Based on the co-transduction data in Tables 2 and 3, *fabD* could lie between *pyrC* and *cat* (see upper portion of Table 5, order II) or to the other side of *cat* away from *pyrC* (Table 5, order I). The relative frequencies of the four recombinant classes in each cross (Table 5) should be inversely related to the minimum number of crossovers involved in their formation. Although the data in Table 5 do not strongly favor order I, the results of both crosses are more consistent with that assignment than with order II. Hence, we have tentatively placed *fabD* to the right of *cat* as shown in Fig. 2.

DISCUSSION

In the course of mapping the *fabD* site, we observed anomalous results for the co-transduction of *fabD*, *cat*, and *purB* with *pyrC* when *pyrC*$^+$ is selected for in strain X7014L or its derivatives by using P1*vir* grown on strain LA2-89. Furthermore, we encountered difficulty obtaining *purB*$^+$ transductants in strain X7014L (*purB51*) and in strain AB740 (*purB15*) but not in strain H680 (*purB51*) with P1*vir* grown on either strain LA2-89 or LA12-G. This latter result was surprising because no difficulties were reported in an earlier study with the selection of *purB*$^+$ in strain X7014L or in the co-transduction between *pyrC* and *cat* or *purB* by using P1 grown on strain LA12-G (22). Since the *fabD* mutations in strains LA2-89 and LA2-130 account for the temperature-sensitive phenotype of these strains (7) and since they can be transferred at the expected frequency to some strains (e.g., H680), it would appear probable that genetic differences between strains LA2-89 or LA2-130 and strain X7014 or its derivatives, that have no known phenotypic

effect, interfere with recombination in a part of the *pyrC* to *purB* region. In the case of strains LA2-89 and LA2-130, this explanation is consistent with the fact that nitrosoguanidine, which has been reported to produce multiple mutations in closely linked genes (1), was used in the generation of the *fabD* mutations. If this is the case, it may be possible to recover the *fabD* mutations free of other alterations by repeated transfer through strain H680 (*purB51*).

The studies in this paper establish the genetic location of the cistron coding for malonyl-coenzyme A-ACP transacylase. *fabD* lies at min 24, an observation that places it about 2 min away from *fabA*, the gene coding for the β,γ-dehydrase required for unsaturated fatty acid synthesis. Evidence is accumulating that indicates that the structural genes for fatty acid biosynthesis are widely distributed on the chromosome of *E. coli* (18; D. F. Silbert, Annu. Rev. Biochem., in press). Two possible exceptions to this conclusion have been noted (D. F. Silbert, Annu. Rev. Biochem., in press), and one of these exceptions involves the *fabD* region. Broekman has described a mutation (*vtr*) which leads in vivo to increased amounts of *cis*-Δ^{11}-18:1 and reduced levels of *cis*-Δ^{9}-16:1 in the phospholipid of the cell (J. H. F. F. Broekman, Ph.D. thesis, Faculty of Science, State University, Utrecht, The Netherlands, 1973). *vtr* is co-transduced with *pyrC* and *purB* at frequencies comparable to those observed for *fabD* (J. H. F. F. Broekman, Ph.D. thesis, 1973). *vtr* and *fabD* may be two separate sites that are closely linked or they may be mutations in the same gene that have different phenotypes. The decrease in malonyl transacylase in *fabD* mutants causes a preferential reduction in vivo in long-chain fatty acid groups of the phospholipid (7). It is possible that other structural alterations in malonyl transacylase might lead to higher rather than lower steady-state levels of malonyl ACP, and consequently enhance rather than reduce the extent of chain elongation. Alternately, since there is recent evidence that demonstrates two distinct β-ketoacyl ACP synthetases in *E. coli*, differing especially in that synthetase II but not synthetase I can catalyze at a normal rate the condensation reaction involving *cis*-Δ^{9}-16:1 ACP (G. D'Agnolo, I. S. Rosenfeld, and P. R. Vagelos, manuscript in preparation and Fed. Proc., **33**:645, 1974), *vtr* could be a mutation that increases the level of activity of synthetase II. No in vitro studies with the *vtr* mutant have been reported. Hence, further genetic and biochemical investigations will be necessary to determine if there are one or more *fab* genes in the region near min 24. It is interesting to note that malonyl ACP, the product of malonyl transacylase, is one of the substrates for the β-ketoacyl synthetases. Thus, there could be functional significance to a clustering of genes coding for these two enzymes.

ACKNOWLEDGMENTS

We thank Barbara Bachmann, Jon Beckwith, John Cronan, Hubert Condamine, John Guest, A. Sasarman, and Bonnie Tyler for their generous gifts of strains. We are very grateful to John Guest for his advice and encouragement with regard to locating the site of the *pyr* mutation in strain AT3143.

This work was supported by U.S. Public Health Service research grant GM 16292 to D. F. Silbert from the National Institute of General Medical Sciences. D. F. Silbert is the recipient of a U. S. Public Health Service Research Career Development award.

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