JOURNAL OF BACTERIOLOGY, June 1976, p. 1351-1354

Vol. 126, No. 3
Printed in U.S.A.

Partial Characterization of a Temperature-Sensitive Mutation Affecting Acetyl Coenzyme A Carboxylase in *Escherichia coli* K-12

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Received for publication 19 March 1976

A temperature-sensitive mutation in *Escherichia coli* K-12 was shown to affect acetyl coenzyme A carboxylase and to map at min 63. This site is designated *fabE*.

In earlier publications we reported on the selection of temperature-sensitive mutants in *Escherichia coli* K-12 affecting fatty acid synthesis (9) and on the characterization of one class (*fabD*) defective in malonyl coenzyme A (CoA)-acyl carrier protein transacylase (10, 15). The subject of this paper is the partial characterization of the enzymatic defect and the chromosomal location of another class of these temperature-sensitive mutants.

The first enzyme in the pathway of fatty acid biosynthesis is acetyl CoA carboxylase, which catalyzes the formation of malonyl CoA from acetyl CoA. In *E. coli* this enzyme is composed of three nonidentical subunits—biotin carboxyl carrier protein (BCCP), biotin carboxylase, and transcarboxylase—that participate in the overall reaction as follows (17):

$$ATP + HCO_3^- + BCCP \xrightarrow{\text{biotin carboxylase}} CO_2^-\text{-}BCCP + ADP + P_i \quad (1)$$

$$CO_2^-\text{-}BCCP + \text{acetyl CoA} \xrightarrow{\text{transcarboxylase}} BCCP + \text{malonyl CoA} \quad (2)$$

Table 1 lists the strains used in this study. Strains L8 and L48 are temperature-sensitive

TABLE 1. *Bacterial strains used*

Strain	Sex	Chromosomal markers[a]	Source
AB1623	F⁻	*thi-1, gltA5, ara-14, lacY1, galK2, xyl-5, mtl-1, tfr-5, tsx-57, str-20*	H. L. Kornberg (4)
AB2834	F⁻	*aroE353, mal-352, tsx-352, supE42(?),* λ⁻	Pittard and Wallace strain (14) via CGSC
CA198	Hfr	*galU106, rel-1,* λ⁻; origin and direction of transfer same as that of HfrH (see reference 12)	S. Brenner strain via CGSC
KL14	Hfr	*thi-1, rel-1,* λ⁻; for origin and direction of transfer, see Low (12)	B. Low (12) via J. Cronan
KL209	Hfr	*supE44, malB16,* λ^{R}λ⁻; for origin and direction of transfer, see Low (12)	B. Low (12) via J. Cronan
L4[b]	F⁻	*fabB, fadE62*	P. Overath (13)
L8[c]	F⁻	*fabE22, thi-1, gltA5, ara-14, lacY1, galK2 xyl-5, mtl-1, tfr-5, tsx-57, str-20*	Harder et al. (9)
L48[d]	F⁻	*fabD1, thi-1, gltA5, ara-14, lacY1, galK2, xyl-5, mtl-1, tfr-5, tsx-57, str-20*	Harder et al. (9, 15)
MA1030	Hfr	*argR64, thi-1, purF1,* λ⁻; a double male with origins and direction of transfer the same as that of HfrH and AB312 (see reference 12)	W. Maas (11)
N728	Hfr	*metB1, purF1, spc-296, nek-728, lacY1 or Z4, xyl-7, mtl-2, gal-6, tonA2, tsx-1,* λ⁻, *supE44;* origin and direction of transfer same as AB312 (see reference 12)	D. Apirion strain from strain JC12 (5) via strain N296 (see reference 6)

[a] Locus designations are those of Taylor and Trotter (16); allele numbers are those of the *E. coli* Genetic Stock Center (CGSC), Department of Microbiology, Yale University, New Haven, Conn.
[b] Identical to K1060.
[c] Formerly LA2-22.
[d] Formerly LA2-89.

mutants obtained by nitrosoguanidine mutagenesis from strain AB1623 (9). The mutants grow normally on minimal medium at 30 C without fatty acid supplement but require fatty acid supplements for prolonged growth at 37 C (9, 10). Strain L48 is one of the mutants whose temperature-sensitive phenotype results from a thermosensitive malonyl CoA-acyl carrier protein transacylase (10). When one compares the properties of cell-free extracts from the mutant (strain L8) and control strains (AB1623 and L48), the following differences are noted: acetyl CoA carboxylase activity is reduced and is temperature sensitive (Fig. 1); biotin carboxylase is also rather thermolabile but its lability only partially accounts for the temperature sensitivity of the overall acetyl CoA carboxylase reaction catalyzed by extracts from strain L8 (Fig. 1). The amount of protein-bound biotin is reduced about 60 to 75% in crude cell or 25 to 45% ammonium sulfate extracts and in an E_a fraction (3) (data not shown; all determinations are based on trichloroacetic acid-precipitable radioactivity in preparations obtained from cells grown with ^3H-labeled biotin). When E_a, a fraction containing biotin carboxylase and BCCP but little transcarboxylase (2, 3), is prepared from normal *E. coli* and added to ammonium sulfate extracts from strain L8 that were inactivated at 42 C, acetyl CoA carboxylase activity is restored to normal levels (Fig. 1). Conversely, there is no effect when E_b, a fraction that contains the transcarboxylase but not the other two components of the acetyl CoA carboxylase complex (1-3), is added to the inactivated extract instead of E_a. Because of the central role of BCCP in the overall reaction, a defect in this component could affect both partial reactions: biotin carboxylation and the transfer of the carboxyl group from carboxyl BCCP to acetyl CoA. Hence, the findings presented here suggest but do not prove that the mutation in strain L8 affects the stability of BCCP.

We have determined that this temperature-sensitive mutation is located on the chromosome of *E. coli* at min 63 and have designated this site as *fabE*. Interrupted matings were performed with strain L8 and strain Hfr KL14 (origin at min 60; clockwise transfer of genes) or Hfr KL209 (origin at min 79; counterclockwise transfer of genes). *fab*$^+$ and *xyl*$^+$ recombinants of strain L8 were selected, and bacteriophage T6 was used to eliminate the donor strain. These crosses indicated that *fabE* was between the origin of strain KL14 (min 60) and *xyl* (min 70) and close to *strA*, since the earliest *fab*$^+$ from the cross with strain KL14 (*strA*$^+$) were *strA* (the recipient allele). Table 2 shows that *fabE*$^+$ co-transduces with *aroE*, *strA*, and *argR* and

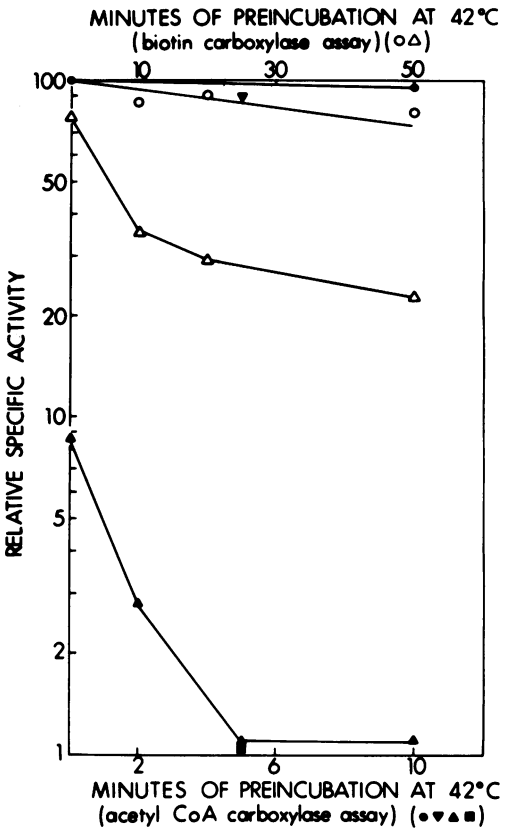

FIG. 1. *Thermolability of acetyl CoA and biotin carboxylase activities in extracts of strain L8. Cells were grown at 30 C as described previously (9). Ammonium sulfate (AS) extracts were prepared as follows: a 0 to 45% AS precipitate was obtained as described elsewhere (8); this fraction was redissolved at 20 mg of protein/ml and the solution was adjusted with AS to 25% saturation; the insoluble material was removed by centrifugation; lastly, the supernatant was brought to 45% saturation with AS and the precipitate was collected. Inactivation of AS extracts at 42 C was conducted in 0.05 M imidazole buffer, pH 6.7, at 20 mg of protein/ml. Acetyl CoA and biotin carboxylase activities were assayed by the radioisotopic procedures of Alberts and Vagelos (3) and of Dimroth et al. (7), respectively; in both assays, $KH^{12}CO_3$ was used at 14 mM (1 μCi/μmol). E_a and E_b were fractions from E. coli B that were generously provided by A. Alberts. Symbols: acetyl CoA (●) and biotin (○) carboxylase activities in 25 to 45% AS extracts from strains AB1623 and L48 (acetyl CoA carboxylase activity for both extracts was measured also after 20, 40, and 80 min and remained essentially at the 100% level [data not shown]); acetyl CoA (▲) and biotin (△) carboxylase activities for 25 to 45% AS extracts from strain L8; acetyl CoA carboxylase activity of inactive (42 C for 5 min) extracts from strain L8 after addition of E_a (▼) or E_b (■) fractions from E. coli B (activity shown with E_a does not include activity of E_a by itself, which was 10% of the total).*

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

Cross no.	Bacterial strains and relevant markers		Marker selected	Colonies with donor marker/total colonies scored	Co-transduction frequency (%)
	Donor	Recipient			
1	AB2834 *aroE353*[b]	L8 *fabE22*	*fabE*$^+$	142/446	31.9
2	MA1030 *argR*[c]	L8 *fabE22*	*fabE*$^+$	47/360	13.1
3	CA198 *strA*$^{+d}$	L8 *fabE22*	*fabE*$^+$	30/253	11.9
4	AB2834 *strA*$^{+d}$	L8 *fabE22*	*fabE*$^+$	21/337	6.2
5	N728 *strA*$^{+d}$	L8 *fabE22*	*fabE*$^+$	28/318	8.8
6	L8 *fabE22*	L4 *strA*$^+$	*strA20*[d]	21/533[e]	4.0

[a] The selection media and transduction procedures have been described elsewhere (9, 15) with the following modifications: bacteriophage were absorbed at 31 C for 20 min; thereafter, the bacteria were plated and in crosses 1 to 5 maintained at 30 C for 60 min before being shifted to 42 C or in cross 6 simply incubated at 30 C.

[b] Selection plates for monitoring co-transduction of *aroE* with *fab*$^+$ contained shikimic acid (10^{-5} M) and phenylalanine, tryptophan, and tyrosine (each at 25 μg/ml).

[c] Inheritance of *argR* was determined by scoring for canavanine resistance as described by Kadner and Maas (11). Plates were examined at 18 h to obtain the clearest results; inhibition of growth of single colonies was the most reliable measure of canavanine sensitivity.

[d] Streptomycin sulfate (200 μg/ml) was used in the solid medium to score for streptomycin sensitivity or to select for streptomycin resistance.

[e] Total of three experiments (2/60, 7/219, 12/254) involving different recipient cultures but the same P1*vir* stock.

that *fabE* can be transferred into other strains by selection for *strA*. Although our initial experiments with this latter cross suggested that *fabE* was transduced poorly, more extensive data indicated that the co-transduction frequency of *fabE* with *strA* averaged about 50% the value obtained in the reciprocal cross (Table 2, compare line 6 with lines 4 and 5). That the expression of *fabE* can be more severe in other genetic backgrounds is suggested by the observation that the thermosensitive phenotype of *fabE* derivatives of strain L4 extended to lower temperatures (by a few degrees Celsius) than that of strain L8 grown under comparable conditions. Hence, the difference between the results shown in line 6 versus lines 4 and 5 (Table 2) might be due to reduced survival of *fabE* transductants. In any case, the linkage data presented here and the fact that *argR* (min 62) has not been shown to co-tranduce with *strA* (min 64) or *aroE* (min 63.7) strongly favor the map order *argR fabE aroE strA*.

We want to thank A. Alberts for the gift of E_a and E_b fractions from *E. coli* B; B. Bachmann, W. Maas, and D. Apirion for providing bacterial strains; R. Ladenson for excellent technical assistance in part of this study; and B. Bachmann for reading the manuscript.

This work was supported by Public Health Service research grant GM-16292 (to D. F. S.) from the National Institute of General Medical Sciences. D. F. S. is the recipient of Public Health Service Research Career Development Award 5-KO4-GM70654 from the National Institute of General Medical Sciences. A. C. received support from Public Health Service predoctoral training grant 5-T01-GM-01311-11 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Alberts, A. W., S. G. Gordon, and P. R. Vagelos.** 1971. Acetyl CoA carboxylase: the purified transcarboxylase component. Proc. Natl. Acad. Sci. U.S.A. 68:1259–1263.
2. **Alberts, A. W., A. M. Nervi, and P. R. Vagelos.** 1969. Acetyl CoA carboxylase. II. Demonstration of biotin-protein and biotin carboxylase subunits. Proc. Natl. Acad. Sci. U.S.A. 63:1319–1326.
3. **Alberts, A. W., and P. R. Vagelos.** 1968. Acetyl CoA carboxylase. I. Requirement for two protein fractions. Proc. Natl. Acad. Sci. U.S.A. 59:561–568.
4. **Ashworth, J. M., H. L. Kornberg, and D. L. Nothmann.** 1965. Location of the structural gene for citrate synthase on the chromosome of *Escherichia coli*. J. Mol. Biol. 11:654–657.
5. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. 36:525–557.
6. **Brown, M. E., and D. Apirion.** 1974. Mapping a cluster of ribosomal genes in *Escherichia coli*. Mol. Gen. Genet. 133:317–327.
7. **Dimroth, P., R. B. Guchhart, E. Stoll, and M. D. Lane.** 1970. Enzymatic carboxylation of biotin: molecular and catalytic properties of a component enzyme of acetyl CoA carboxylase. Proc. Natl. Acad. Sci. U.S.A. 67:1353–1360.
8. **Fall, R. R., and P. R. Vagelos.** 1972. Acetyl CoA carboxylase. Molecular forms and subunit composition of biotin carboxyl carrier protein. J. Biol. Chem. 247:8005–8015.
9. **Harder, M. E., I. R. Beacham, J. E. Cronan, Jr., K. Beacham, K. L. Honegger, and D. F. Silbert.** 1972. Temperature-sensitive mutants of *Escherichia coli* requiring saturated and unsaturated fatty acids for growth: isolation and properties. Proc. Natl. Acad. Sci. U.S.A. 69:3105–3109.
10. **Harder, M. E., R. C. Ladenson, S. D. Schimmel, and D. F. Silbert.** 1974. Mutants of *Escherichia coli* with temperature-sensitive malonyl coenzyme A-acyl carrier protein transacylase. J. Biol. Chem. 349:7468–7475.
11. **Kadner, R. J., and W. K. Maas.** 1971. Regulatory gene mutations affecting arginine biosynthesis in *Escherichia coli*. Mol. Gen. Genet. 111:1–14.
12. **Low, B..** 1973. Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. J. Bacteriol. 113:798–812.
13. **Overath, P., H. U. Schairer, and W. Stoffel.** 1970.

Correlation of *in vivo* and *in vitro* phase transitions of membrane lipids in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. 67:606–612.

14. **Pittard, J., and B. J. Wallace.** 1966. Distribution and function of genes concerned with aromatic amino acid biosynthesis in *Escherichia coli*. J. Bacteriol. 91:1494–1508.
15. **Semple, K. S., and D. F. Silbert.** 1975. Mapping of the *fabD* focus for fatty acid biosynthesis in *Escherichia coli*. J. Bacteriol. 121:1036–1046.
16. **Taylor, A. L., and C. D. Trotter.** 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. 36:504–524.
17. **Volpe, J. J., and P. R. Vagelos.** 1973. Saturated fatty acid biosynthesis and its regulation. Annu. Rev. Biochem. 42:21–60.