

The *dedB* (*usg*) Open Reading Frame of *Escherichia coli* Encodes a Subunit of Acetyl-Coenzyme A Carboxylase

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In the course of work on *Escherichia coli* acetyl-coenzyme A (acetyl-CoA) carboxylase (9), we noted a strong amino acid sequence similarity between the β subunit of rat propionyl-CoA carboxylase and the predicted protein product (304 residues) of an *E. coli* open reading frame of unknown function previously called *dedB* (for DNA downstream of *hisT*; 11) and *usg* (for gene upstream of *folC*; 4) (Fig. 1). Further analysis showed that the predicted DedB/Usg protein also had sequence similarity to the 12S subunit of the *Propionibacterium shermanii* transcarboxylase (Fig. 1). *E. coli* acetyl-CoA carboxylase is composed of two components, biotin carboxylase and carboxyltransferase, which catalyze distinct half-reactions; (i) the carboxylation of biotin with bicarbonate, followed by (ii) transfer of the CO₂ group from carboxybiotin to acetyl-CoA to form malonyl-CoA (1, 12). A third subunit, biotin carboxyl carrier protein (BCCP), carries the essential biotin cofactor covalently bound to a lysine residue proximal to its carboxyl terminus.

The reactions catalyzed by the rat and *P. shermanii*

proteins are mechanistically related to that catalyzed by acetyl-CoA carboxylase (7, 13). Propionyl-CoA carboxylase catalyzes a reaction closely analogous to that catalyzed by acetyl-CoA carboxylase (given the substitution of propionate for acetate). The rat enzyme is composed of two subunits (α and β). The α subunit contains sequences having extensive similarity to *E. coli* biotin carboxylase and BCCP (9). Thus, the similarity between the DedB/Usg protein and the rat β subunit strongly suggested that the *dedB/usg* gene product is a component catalyzing the carboxyltransfer half-reaction. This interpretation is consistent with the sequence similarity to the *P. shermanii* 12S sequence, which catalyzes a carboxyltransferase reaction (7, 13).

We report here that a temperature-sensitive mutant known to be blocked early in the fatty acid synthetic pathway (6, 14) is an allele of the *dedB/usg* gene (Fig. 2) and encodes a defective carboxyltransferase. Mutant strain LA1-6 was isolated in 1970 by Harder et al. (6) by a tritiated acetate suicide selection. This mutant has a temperature-sensitive

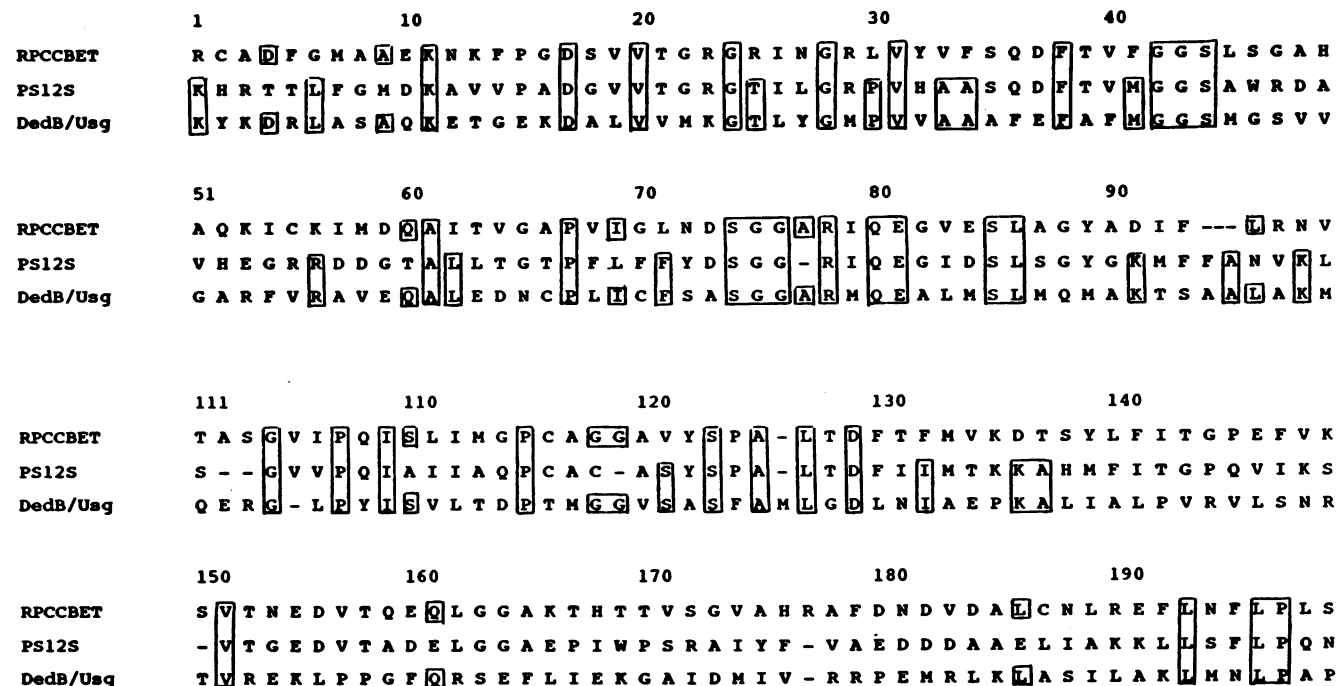


FIG. 1. Similarities between DedB/Usg and known biotin carboxylase enzymes. Comparisons of DedB/Usg with residues 91 to 283 of the β subunit of rat propionyl-CoA carboxylase (RPCCBET; 8) and/or residues 67 to 255 of the 12S subunit of *P. shermanii* transcarboxylase (PS12S; 13) are shown. Amino acid residues found in both DedB/Usg (residues 90 to 283; 4, 11) and at least one of the two known carboxylase sequences are boxed.

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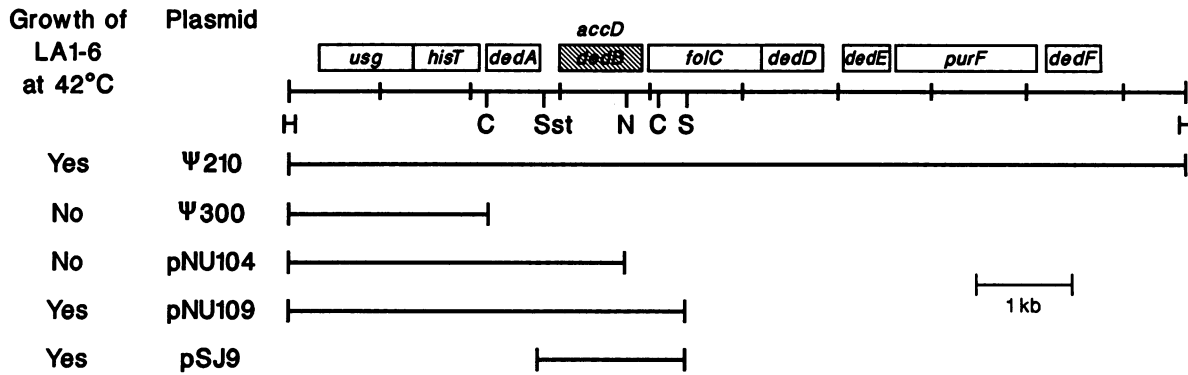


FIG. 2. Restriction map of the min-50 region and assay of the abilities of various recombinant plasmids to complement the temperature-sensitive growth phenotype of strain LA1-6. The chromosomal segment shown is located at 2440.97 kbp on the *E. coli* physical map (10). The locations of key restriction sites (see reference 11 for a more detailed map), plasmid designations, and complementation abilities are shown. All chromosomal segments were cloned into plasmid pBR322. Restriction sites: H, *Hind*III; C, *Cla*I; Sst, *Sst*II; N, *Nru*I; and S, *Sal*I. All plasmids except pSJ9 were the gifts of M. Winkler (2). Plasmid pSJ9 was constructed by digestion of plasmid pNU109 with *Sst*II and *Hind*III followed by digestion of the protruding ends with mung bean nuclease and recircularization of the plasmid with phage T4 DNA ligase.

lesion early in the fatty acid biosynthetic pathway and a phenotype virtually identical to that of another strain, LA2-89 (also called L8), isolated in the same selection, which encodes a mutant BCCP defective in biotin acceptor activity (6, 9). The lesion in strain LA1-6 (also called *fab6*) was mapped to the min-50 region of the *E. coli* chromosome (14), where the *dedB/usg* gene is located (2, 4, 11) (2440.97 kbp on the physical map; reviewed in reference 10). It therefore seemed likely that the mutational lesion of strain LA1-6 was within the *dedB/usg* gene. This was demonstrated by complementation of the temperature-sensitive growth of strain LA1-6 by plasmids containing cloned chromosomal fragments in which *dedB/usg* was the only common gene (Fig. 2). We also demonstrated that the carboxyltransferase component of strain LA1-6 was defective. While strain W3110 showed 77 U of carboxyltransferase activity per mg of protein (1 U is equal to the formation of 1 nmol of carboxybiotin per min) when assayed at 30°C on ammonium sulfate-purified extracts as described by Guchhait et al. (5), carboxyltransferase activity could not be detected in extracts of strain LA1-6 grown and assayed at 30°C. This suggested that the mutant enzyme is unusually labile in vitro. A mixture of enzyme preparations from strain W3110 (12.5 µg of protein) and strain LA1-6 (7.2 µg of protein) showed 57 U of activity per mg. Introduction of plasmid pSJ9, which contains the *dedB/usg* gene cloned into plasmid pBR322, restored carboxyltransferase activity (145 U/mg). These data demonstrate that the *dedB/usg* gene encodes a carboxyltransferase subunit.

The carboxyltransferase component of *E. coli* acetyl-CoA carboxylase consists of two subunit proteins of 35 and 30 kDa in a 1:1 complex (5), whereas the *dedB/usg* open reading frame encodes a protein of 33 kDa. The DedB/UsG protein observed in maxicells migrates as a 33 kDa protein (3, 11); thus, aberrant migration on sodium dodecyl sulfate gels cannot explain the discrepancy. It seems likely that the 30-kDa subunit is the DedB/UsG protein and that the lower molecular weight is due to proteolysis during purification, since proteolysis during the parallel purification of BCCP was reported (5). It is also possible that the 30-kDa protein is a proteolytic degradation product of the 35-kDa protein, but we favor the possibility that these proteins are products of two different genes. This premise is based on the data of Guchhait et al. (5) and our finding that the presence of a

functional *dedB/usg* gene on a multicopy plasmid gave only a twofold overproduction of carboxyltransferase activity, suggesting that another component (e.g., the 35-kDa subunit) was limiting. Moreover, the sequence similarity between the DedB/UsG protein and the rat propionyl-CoA β subunit is restricted to the amino terminus of the latter protein, and the DedB/UsG protein lacks an acyl-CoA binding motif (13). Hence, we suggest that the 35-kDa protein will be similar to the carboxyl half of the rat β subunit and will contain an acetyl-CoA binding site. The location of the gene encoding the 35-kDa subunit remains to be determined.

The *dedB/usg* gene must be renamed. We have assigned the designations *accB* and *accC* (for acetyl-CoA carboxylase) to the BCCP and biotin carboxylase subunits (9), respectively. We shall retain the *accA* designation for the gene encoding the acetyl-CoA binding carboxyltransferase subunit (the 35-kDa protein) and therefore assign *accD* to the gene formerly called *dedB* and *usg*.

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REFERENCES

- Alberts, A., and P. R. Vagelos. 1972. Acyl-CoA carboxylases, p. 37-82. In P. D. Boyer (ed.), *The enzymes*, 3rd ed., vol. 6. Academic Press, New York.
- Arps, P. J., and M. E. Winkler. 1987. Structural analysis of the *Escherichia coli* K-12 *hisT* operon by using a kanamycin resistance cassette. *J. Bacteriol.* **169**:1061-1070.
- Bognar, A., C. Pyne, M. Yu, and G. Basi. 1989. Transcription of the *folC* gene encoding folylpolyglutamate synthetase-dihydrofolate synthetase in *Escherichia coli*. *J. Bacteriol.* **171**:1854-1861.
- Bognar, A. L., C. Osborn, and B. Shane. 1987. Primary structure of the *Escherichia coli folC* gene and its folylpolyglutamate synthetase-dihydrofolate synthetase product and regulation of expression by an upstream gene. *J. Biol. Chem.* **262**:12337-12343.
- Guchhait, R. B., S. E. Polakis, P. Dimroth, E. Stoll, J. Moss, and M. D. Lane. 1974. Acetyl-CoA carboxylase system of *Escherichia coli*: purification and properties of the biotin carboxylase, carboxyltransferase, and carboxyl carrier proteins. *J. Biol. Chem.* **249**:6633-6645.
- Harder, M. E., I. R. Beacham, J. E. Cronan, Jr., K. Beacham, J. 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. USA **69**:3105–3109.
7. Knowles, J. R. 1989. The mechanism of biotin-dependent enzymes. Annu. Rev. Biochem. **58**:195–222.
 8. Kraus, J. P., F. Firgauci, J. Novotny, F. Kalousek, K. R. Williams, C. Williamson, T. Ohina, and L. E. Rosenberg. 1986. Coding sequence of the precursor of the β subunit of rat propionyl-CoA carboxylase. Proc. Natl. Acad. Sci. USA **83**:8049–8053.
 9. Li, S. J., and J. E. Cronan, Jr. 1992. The gene encoding the biotin carboxylase subunit of *Escherichia coli* acetyl-CoA carboxylase. J. Biol. Chem. **267**:855–863.
 10. Médigue, C., A. Viari, A. Hénaut, and A. Danchin. 1991. *Escherichia coli* molecular genetic map (1500 kbp) update II. Mol. Microbiol. **5**:2629–2640.
 11. Nonet, M. L., C. C. Marvel, and D. R. Tolan. 1987. The *hisT-purF* region of the *Escherichia coli* K-12 chromosome. J. Biol. Chem. **262**:12209–12217.
 12. Polakis, S. E., R. B. Guchhait, E. E. Zwergel, M. D. Lane, and T. G. Cooper. 1974. Acetyl-CoA carboxylase system of *Escherichia coli*: studies on the mechanisms of the biotin carboxylase and carboxyltransferase catalyzed reactions. J. Biol. Chem. **249**:6657–6667.
 13. Samols, D., C. G. Thornton, V. L. Murtif, G. K. Kumar, F. C. Haase, and H. G. Wood. 1988. Evolutionary conservation among biotin enzymes. J. Biol. Chem. **263**:6461–6464.
 14. Silbert, D. F., J. E. Cronan, Jr., I. R. Beacham, and M. F. Harder. 1974. Genetic engineering of membrane lipid. Fed. Proc. **33**:1725–1732.