The fadD gene of Escherichia coli encodes the inner-membrane-associated acyl coenzyme A synthetase (acyl-CoA synthetase) [fatty acid:CoA ligase (AMP-forming), EC 6.2.1.3] (6). Acyl-CoA synthetases catalyze the formation of fatty acyl-CoA by a two-step mechanism that proceeds through the pyrophosphorolysis of ATP (2).

\[
\text{Mg}^{2+} + \text{Fatty acid} + \text{ATP} \rightarrow [\text{Fatty acid-AMP}] + \text{PP} + \text{CoASH} \rightarrow \text{Fatty acid-SCoA} + \text{AMP}
\]

E. coli contains a single acyl-CoA synthetase, which has been purified to homogeneity (3, 4). In the process of long-chain fatty acid transport, this enzyme plays a pivotal role by catalyzing the thioesterification of exogenous fatty acids into metabolically active CoA thioesters prior to β-oxidation concomitant with transport. This enzyme has broad chain-length specificity, giving \( V_max \) values ranging from 2,632 nmol/min/mg of protein for lauric acid (C₁₂) to 135 nmol/min/mg of protein for hexanoate (C₆) (3). Maximal activities associated with this enzyme are found with fatty acids ranging in length from C₁₂ to C₁₈ (3).

The structural gene for acyl-CoA synthetase (fadD) was identified by Overath et al. (6), who mapped this locus to the 40-min region of the E. coli chromosome. I have used 11 clones representing the 40-min region of the E. coli chromosome from the Kohara library (5) (clones 5E12, 4B8, 12H7, 3E12, 9F2, 7F2, 6D1, 12B3, 15D5, 19H3, and 12C7) to fine map and subclone the fadD gene (1). The fadD88 strain PN235 was transduced with these clones along with λC857 as a helper phage and then plated on oleate minimal agar plates. After 72 h at 30°C, dilsyogens that were able to grow on oleate as a sole carbon and energy source (Ole⁺) were identified in cells infected with clones 7F2 and 6D1. A second round of lysogenic complementation demonstrated that clones 7F2 and 6D1 were both able to confer an Ole⁺ phenotype in the fadD88 strain PN235. Clone 6D1 was used as a source of DNA to subclone the fadD gene for further analysis (1). Analysis using restriction endonucleases indicated that the fadD gene is located at approximately 1905 kb, corresponding to 39.5 min on the genetic map (Fig. 1).

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