Fatty Acid Degradation in Caulobacter crescentus

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Fatty acid degradation was investigated in Caulobacter crescentus, a bacterium that exhibits membrane-mediated differentiation events. Two strains of C. crescentus were shown to utilize oleic acid as sole carbon source. Five enzymes of the fatty acid β-oxidation pathway, acyl-coenzyme A (CoA) synthase, crotonase, thiolase, β-hydroxyacyl-CoA dehydrogenase, and acyl-CoA dehydrogenase, were identified. The activities of these enzymes were significantly higher in C. crescentus than the fully induced levels observed in Escherichia coli. Growth in glucose or glucose plus oleic acid decreased fatty acid uptake and lowered the specific activity of the enzymes involved in β-oxidation by 2- to 3-fold, in contrast to the 50-fold glucose repression found in E. coli. The mild glucose repression of the acyl-CoA synthase was reversed by exogenous dibutyryl cyclic AMP.

Acyl-CoA synthase activity was shown to be the same in oleic acid-grown cells and in cells grown in the presence of succinate, a carbon source not affected by catabolite repression. Thus, fatty acid degradation by the β-oxidation pathway is constitutive in C. crescentus and is only mildly affected by growth in the presence of glucose. Tn5 insertion mutants unable to form colonies when oleic acid was the sole carbon source were isolated. However, these mutants efficiently transported fatty acids and had β-oxidation enzyme levels comparable with that of the wild type. Our inability to obtain fatty acid degradation mutants after a wide search, coupled with the high constitutive levels of the β-oxidation enzymes, suggest that fatty acid turnover, as has proven to be the case in fatty acid biosynthesis, might play an essential role in membrane biogenesis and cell cycle events in C. crescentus.

The cell cycle of the gram-negative bacterium Caulobacter crescentus exhibits a series of unicellular differentiation events. Several of these events involve changes at the cell surface and include the biogenesis of a polar flagellum, chemosensory receptors, phage receptors, and pili (23). The synthesis of many of the membrane proteins involved in these structures and functions is temporally regulated. Upon synthesis these proteins are localized to specific portions of the cell and segregate to only one daughter cell upon division (23). Thus, some type of spatial control mechanism must contribute to their positioning in the cell membrane. To help define the role that the cell membrane might play in these processes, we investigated the composition and metabolism of the membrane phospholipids.

The negatively charged phospholipids, phosphatidylycerol and cardiolipin, are the major components, and neither phosphatidylethanolamine nor phosphatidylserine can be detected in C. crescentus membranes (6). The enzymatic pathway of phospholipid synthesis in C. crescentus is the same as the comparable portions of the pathway in Escherichia coli (5). The fatty acid components of the phospholipids consist of both saturated and unsaturated 14-, 16-, and 18-carbon fatty acids (4, 16), and these fatty acids are synthesized by the bacterial anaerobic pathway in which a double bond is introduced on a 10-carbon chain during chain elongation (16). We have also demonstrated that in wild-type strains of C. crescentus, in contrast to E. coli, exogenous fatty acids can be incorporated intact into phospholipid (16). Exogenous fatty acids repressed the synthesis of new fatty acids and induced the turnover of endogenous fatty acids from phospholipid (16).

Based on the pathways of biosynthesis and catabolism of these lipids, we have attempted to isolate mutants altered in membrane biogenesis and to then use these mutants to study the relationship between membrane biogenesis and the temporal and spatial regulation of surface differentiation events (5, 7, 11–13, 24). Two C. crescentus strains auxotrophic for fatty acids, carrying mutations in faA and faB, respectively, were isolated and found to be altered in some aspect of a regulatory link between membrane synthesis and cell cycle events (11–13). Although a large number of auxotrophs were isolated, none were due to mutations in the enzymes of the biosynthetic pathway, suggesting that the link between membrane lipid metabolism and cell cycle events might result in indispensable functions for the enzymes involved in these pathways.

We investigated the pathway of fatty acid catabolism and its regulation in an effort to understand fatty acid utilization and turnover in C. crescentus. We report here that C. crescentus can utilize fatty acids as sole carbon source and that the five enzymes shown in E. coli to participate in fatty acid β-oxidation are present in C. crescentus at high constitutive levels. The activity of these enzymes was decreased two- to threefold in cells grown in the presence of glucose. The mild glucose effect was reversed by dibutyryl cyclic AMP (cAMP). This apparently weak enzyme modulation is in contrast to that found in E. coli, in which the β-oxidation enzymes are induced by exogenous fatty acids and are repressed between 50- and 100-fold by glucose (20, 27).

As in the case of earlier attempts to isolate mutants in the pathway of fatty acid biosynthesis, a large number of mutants unable to use fatty acids as sole carbon source were isolated, but none of these proved to be altered in fatty acid uptake or in the enzymes of the β-oxidation pathway.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study were the wild-type strain Caulobacter crescentus CB13 and a proline auxotroph of C. crescentus...
CB15 (SC451). They were grown at 30°C in peptone yeast extract broth (22), minimal medium (BM) (16) supplemented with (0.2%) glucose (BMG), or when appropriate with 1% Tergitol Nonidet P-40 (NP-40) plus 1 mM oleic acid (BMT0) (16). E. coli Le392, kindly provided by W. Nunn, was grown at 37°C in LB medium or in M-9 minimal medium supplemented with either 0.5% glucose or 1% Tergitol plus 1 mM oleic acid.

Cells were shifted from one medium to another by collection on sterile 0.45-μm-pore-size membrane filters (Millipore Corp., Bedford, Mass.).

**Mutant isolation.** Tn5 insertion mutants were derived from *C. crescentus* SC451 by mating with *E. coli* AEEI03, which harbors the plasmid pH1J1 (8). This plasmid contains a Tn5 insert that transposes randomly into the *C. crescentus* chromosome and is stably maintained. Mutants in fatty acid utilization were selected by their inability to form colonies on minimal media plates containing oleic acid (1 mM) as sole carbon source and by their Tn5-encoded resistance to kanamycin. Of 4,000 kanamycin-resistant mutants, 12 were unable to form colonies when oleic acid was available as sole carbon source.

**Materials.** [14C]Oleic acid was obtained from New England Nuclear Corp. (Boston, Mass.). Oleic acid was obtained from Fisher Scientific Co. (Pittsburgh, Pa.). [14C]Decanoic acid was obtained from ICN Pharmaceuticals Inc. (Irvine, Calif.). Crotonyl-coenzyme A (CoA) and CoA were obtained from P-L Biochemicals, Inc. (Milwaukee, Wis.). ATP and NADH were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.).

**Enzyme assays.** Cultures of *C. crescentus* or *E. coli* were harvested at the early log phase of growth and suspended in 5 ml of 10 mM KH2PO4 (pH 7.5). Crude extracts were prepared by disruption in a French press, as described by Maloy et al. (18). The cell lysate was centrifuged at 15,000 × g for 30 min at 4°C, and the protein content of the supernatant was determined by the procedure described by Lowry et al. (17).

Acyl-CoA synthase was assayed as described by Komeda and Nunn (15) with [1-14C]oleic acid as substrate. Crotonase was assayed as described by Binstock and Schultz (2) with crotonyl-CoA as substrate. Hydroxyacyl-CoA dehydrogenase was assayed as described by Weeks et al. (27) with acetoacetyl-CoA as substrate. Thiolase was assayed as described by Feigenbaum and Schulz (9) with acetoacetyl-CoA as substrate.

Acyl-CoA dehydrogenase, assayed as described by Binstock et al. (1) with palmitoyl-CoA as substrate, was partially purified prior to assay to eliminate a competing dehydrogenase activity (10). Cell lysates were prepared as described above, and the supernatant from the 15,000 × g spin was centrifuged at 120,000 × g for 120 min. Ammonium sulfate (0.243 g/ml) was added to this supernatant, and the resulting 0 to 40% ammonium sulfate precipitate, which contained all the enzyme activity, was dialyzed overnight against 10 mM KH2PO4.

**Assay of fatty acid uptake.** Fatty acid uptake was determined by measuring the incorporation of exogenously labeled [1-14C]oleic acid into acid-precipitable material. *C. crescentus* SC451 was grown overnight in minimal medium (BM) with glucose (0.2%) (BMG), glucose (0.2%) plus Tergitol (1%) and oleic acid (1 mM) (BMT0), or Tergitol (1%) and oleic acid (1 mM) alone (BMT0). Cultures were grown to a density of approximately 2 × 108 cells/ml, harvested by centrifugation at 15,000 × g for 5 min, washed with BM medium, and centrifuged again for 5 min at 15,000 × g. The cultures were incubated at 30°C in the absence of a carbon source for 30 min. Carbon source (glucose, glucose plus Tergitol and oleic acid, or Tergitol and oleic acid) was then added to the appropriate cultures in the presence of [1-14C]oleic acid (2 μCi/μmol). Duplicate samples (0.5 ml) were removed after various times of incubation, and trichloroacetic acid (10%) was added to each sample. The precipitated material was collected on glass filters (Enzo Biochem, Inc., New York) and washed with 2 ml of BM plus Tergitol (1%) and oleic acid (1 mM). Filters were air dried and counted in liquid scintillation fluid (Hydroflor; New England Nuclear Corp., Boston, Mass.).

Transport of oleic acid into *C. crescentus* parent and mutant strains was also assayed by the steady-state incorporation of [1-14C]oleic acid into lipids. The mutants were grown overnight in BMTO in the presence of [1-14C]oleic acid (2 μCi/μmol). Cultures were harvested at the mid-log phase of growth and washed once with BMTO and then three times in BM. After each wash the cells were collected by centrifugation at 15,000 × g for 5 min. Lipids were extracted by the method of Bligh and Dyer (3), as modified by Contreras et al. (6) and dried under nitrogen. Samples were counted in scintillation fluid (Betaflow; New England Nuclear Corp.).

**RESULTS**

**Growth on exogenous fatty acids.** *C. crescentus* was able to grow in the presence of oleic acid, a monounsaturated fatty acid, as sole carbon source with a doubling time of 7 h (Fig. 1). In the presence of glucose as sole carbon source the rate of growth was at least twice as fast as growth on oleic acid. The total number of viable cells and the optical density of the cultures was comparable in both oleic acid- and glucose-supplemented media. Tergitol, the detergent used to solubilize fatty acids, was not utilized as a carbon source (Fig. 1), and its addition to a glucose-containing medium was shown previously not to inhibit growth (7). *C. crescentus* was also able to use stearic and palmitic acids, both saturated fatty acids, as carbon sources when grown on agar plates.

The rate of [1-14C]oleic acid uptake was measured by its incorporation into acid-precipitable material (Fig. 2). The rate was linear for 15 min in a culture that was grown in the presence of oleic acid as sole carbon source (Fig. 2). A twofold decrease in [14C]oleic acid incorporation was observed if the cells were grown in the presence of both oleic acid and glucose, or in the presence of glucose alone. It therefore appears that the presence of glucose as an alternate carbon source results in a slower velocity of uptake of exogenous fatty acid into the cell.

**Effect of carbon source on acyl-CoA synthase activity.** Acyl-CoA synthase plays a key role in bacterial fatty acid metabolism (14, 15). The enzyme catalyzes the addition of a hydrophobic CoA group to fatty acids to yield an activated substrate for subsequent β-oxidation or incorporation into phospholipids. Four bacterial enzymes work in series to effect the oxidation of fatty acid acyl-CoA to CO2. These are crotonase (enoyl-CoA-hydratase), thiolase, β-hydroxyacyl-CoA dehydrogenase, and acyl-CoA dehydrogenase. The acyl-CoA synthase (Tables 1 and 2) and the four enzymes of the β-oxidation pathway (Table 3) were all detected in two different strains of *C. crescentus*.

To determine whether the activity of acyl-CoA synthase was affected by carbon source, the requirements of the reaction were defined (Table 1). The enzyme was dependent
on Mg²⁺, and this requirement was only partially satisfied by Mn²⁺. The reaction exhibited a slight requirement for Triton X-100, which may indicate membrane association. There was an absolute requirement for CoA and ATP. Decanoic acid could be substituted for oleic acid as the substrate without any decrease in the amount of product formed, indicating that in crude extracts acyl-CoA synthase activity can add CoA to both medium and long-chain fatty acids. The optimum reaction temperature was 40°C, and the enzyme retained half of its activity at 60°C. There was a broad pH optimum for acyl-CoA synthase, with maximum activity at pH 8.

Measurement of acyl-CoA synthase activity in crude extracts of C. crescentus SC451, C. crescentus CB13, and E. coli Le392 grown in oleic acid as sole carbon source (Table 2) revealed that the C. crescentus extracts had a somewhat higher specific activity than the E. coli extract. Extracts of cultures grown with glucose as sole carbon source or with a combination of glucose and oleic acid showed a 2- to 3-fold reduction in enzyme activity in the case of the C. crescentus strains and a 30-fold reduction in the case of E. coli. The mild repression by glucose of the C. crescentus enzyme was reversed by exogenous dibutyryl cAMP, as has been shown to be the case for the stronger glucose repression in E. coli (21). The addition of dibutyryl cAMP relieved most of the reduction in enzyme activity observed in cells growing on glucose as sole carbon source. The specific activity of the acyl-CoA synthase in C. crescentus was comparable in oleic acid-grown cultures and in cultures grown on succinate, a carbon source not affected by catabolite repression. These results suggest that in C. crescentus a major enzyme in the initiation of the β-oxidation pathway is not induced by the fatty acid substrate. In contrast, the E. coli enzyme was induced four- to fivefold by growth on oleic acid (Table 2). Results of both of these experiments and those reported by Overath et al. (20) demonstrate that the E. coli acyl-CoA synthase activity is low in succinate-grown cells and is induced by growth on fatty acids.

Effect of glucose on fatty acid β-oxidation enzyme activities in C. crescentus and E. coli. The activities of crotonase, β-hydroxyacyl-CoA dehydrogenase, and acyl-CoA dehydrogenase were approximately fivefold higher in C. crescentus SC451 than in E. coli (Table 3). The induced level of specific activity of the C. crescentus acyl-CoA synthase was approximately twice that found in E. coli (Table 2). Thiolase had 30-fold higher activity in C. crescentus SC451 and 15-fold higher activity in strain CB13 than the E. coli enzyme level. The specific activities of the four E. coli β-oxidation enzymes shown in Table 3 are comparable within a twofold range to those reported for E. coli enzymes by other workers (25, 27). The C. crescentus strains exhibited a significant but moderate decrease in specific activity of all four enzymes when grown in the presence of glucose. For example, growth in glucose caused a 50-fold reduction in crotonase activity in E. coli, whereas in C. crescentus SC451 there was only a 3-fold reduction in activity. Similar differences in glucose effect were seen with the acyl-CoA synthase (Table 2). Relatively high levels of acyl-CoA dehydrogenase were measured in C. crescentus SC451, whereas both the CB13 strain and E. coli had approximately 10-fold lower enzyme activity. However, the activity of this enzyme was only decreased twofold in both C. crescentus and E. coli grown in the presence of glucose.

Isolation of Tn5 insertion mutants unable to grow on oleic acid as sole carbon source. Twelve independent Tn5 insertion mutants were isolated that were unable to form colonies when oleic acid was the sole carbon source. When replica plated onto medium containing glucose, ribose, xylose, aspartate, lactose, acetate, or succinate as sole carbon source, the mutants could be grouped into two classes. Seven mutants were unable to use oleic acid, xylose, aspartate, lactose, acetate, or succinate as carbon source, suggesting that their lesion was probably in a pivotal point in intermediary metabolism. The remaining five mutants, AE4000, AE4002, AE4010, AE4011, and AE4016, were unable to form colonies only when oleic acid was the sole carbon source. These mutants appear to be bradytrophs, as they grew poorly in the presence of oleic acid in liquid medium, with a generation time that is four to five times longer than that of the parent strain. When glucose was used
TABLE 1. Requirements for acyl-CoA synthase activity in extracts of C. crescentus

| Additions or omissions | Activity⁵ |
|------------------------|-----------|
| Complete               | 100       |
| Omit MgCl₂             | 0.05      |
| Omit MgCl₂ and add 8 mM MnCl₂ | 48       |
| Omit Triton X-100      | 81        |
| Omit ATP               | 1         |
| Omit CoA               | 3         |
| [1-¹⁴C]oleic acid⁴     | 100       |

⁴ Cell extracts of C. crescentus SC451 grown in minimal medium with oleic acid as sole carbon source were prepared as described in the text.
⁵ The complete reaction mixture incorporated 3 nmol/min per mg of protein using the assay described in the text. The activities under the various reaction conditions are shown as a percentage of the complete reaction.

The activities of the five enzymes of the fatty acid degradative pathway in C. crescentus, the acyl-CoA synthase, and the four enzymes which catalyze the β-oxidation of fatty acids, crotonase, thiolase, β-hydroxyacyl-CoA dehydrogenase, and acyl-CoA dehydrogenase, were found to be significantly higher than the fully induced levels observed in E. coli. It appears that in C. crescentus high levels of β-oxidation activity do not require fatty acid induction. This conclusion was supported by the observation that the activity of acyl-CoA synthase in C. crescentus grown in oleic acid was the same as that in succinate-grown cells, whereas in E. coli the acyl-CoA synthase activity was sixfold lower in cells

DISCUSSION

The activities of the five enzymes of the fatty acid degradative pathway in C. crescentus, the acyl-CoA synthase, and the four enzymes which catalyze the β-oxidation of fatty acids, crotonase, thiolase, β-hydroxyacyl-CoA dehydrogenase, and acyl-CoA dehydrogenase, were found to be significantly higher than the fully induced levels observed in E. coli. It appears that in C. crescentus high levels of β-oxidation activity do not require fatty acid induction. This conclusion was supported by the observation that the activity of acyl-CoA synthase in C. crescentus grown in oleic acid was the same as that in succinate-grown cells, whereas in E. coli the acyl-CoA synthase activity was sixfold lower in cells

TABLE 2. Acyl-CoA synthase activity in extracts of C. crescentus⁴° and E. coli⁴ grown on various carbon sources

| Carbon source    | C. crescentus | E. coli |
|------------------|--------------|--------|
| SC451            | CB13         |        |
| Oleic acid       | 2.30         | 0.97   |
| Glucose          | 1.20         | 0.03   |
| Glucose + oleic acid | 1.11       | 0.05   |
| Glucose + dibutyryl cAMP | 2.00⁶ | ND⁴  |
| Glucose + oleic acid + dibutyryl cAMP | 2.38⁶ | ND  |
| Succinate        | 2.63         | ND     | 0.18  |
| Xylose           | 2.00         | ND     |        |

⁴° C. crescentus SC451 and CB13 were grown in minimal medium BM supplemented with 1 mM oleic acid, glucose, succinate, or xylose.
⁴ E. coli Le392 was grown in minimal medium M9 supplemented with 1 mM oleic acid, glucose, or succinate.
⁶ Cultures of C. crescentus SC451 were grown in the presence of 1 mM dibutyryl cAMP plus glucose or glucose and oleic acid.
⁴ ND, Not done.
grown on succinate as opposed to oleic acid (Table 2). These results suggest that in C. crescentus the fatty acid degradative pathway is constitutive.

The constitutive nature of fatty acid degradation in C. crescentus is somewhat surprising given our earlier finding (11) that the rate of turnover of endogenous fatty acids is increased in cells grown in the presence of oleic acid. However, the turnover experiments measured only endogenously synthesized fatty acids that were already acylated to phospholipids. Thus, the turnover experiments necessarily reflected the rate of removal of fatty acids from phospholipids, as well as the rate of degradation of the fatty acids themselves. We have previously demonstrated that exogenous fatty acids can be incorporated directly into phospholipids (16), or they can enter the fatty acid degradation pathway. How the pools of fatty acids destined for these two alternative fates are related to each other is unknown. However, the results reported here suggest that increased turnover of endogenous lipids in cells grown in the presence of oleic acid is not due to increased fatty acid degradation and that some other aspect of lipid metabolism might be regulated in this case.

The high constitutive level of the five β-oxidation enzymes in C. crescentus was reduced only two- to threefold when cells were grown in the presence of glucose. Under compatible conditions, glucose affected a 30- to 50-fold reduction in the specific activity of the E. coli β-oxidation enzymes (Tables 2 and 3) (20, 25). The five β-oxidation enzymes in E. coli are encoded by genes (fad) at four distinct loci on the E. coli chromosome and form a regulon (26). The glucose repression of the E. coli fad regulon is reversed by cAMP (21). Similarly, the mild glucose repression of acyl-CoA synthase in C. crescentus was reversed by the addition of exogenous dibutyl cAMP (Table 2).

The relatively unmodulated and high specific activity of the fatty acid degradation enzymes observed in two different strains of C. crescentus may reflect the fact that these organisms normally grow in a dilute aquatic environment and do not experience the “feast or famine” nutritional environment of the enteric bacteria. Another gram-negative bacterium, the enterobacterium Yersinia pestis, has high basal levels of at least one of the β-oxidation enzymes, the β-hydroxyacyl-CoA dehydrogenase (19). As was observed with C. crescentus, glucose-grown cultures of Y. pestis exhibit only a twofold reduction of β-oxidation enzyme activities. However, the high basal level of activity was induced at least 10-fold in cells grown in the presence of fatty acid.

Mutants unable to form colonies when oleic acid was used as sole carbon source were isolated following Tn5 mutagenesis of C. crescentus. Of the 4,000 colonies that were screened, only 12 were unable to form colonies when supplied with oleic acid. Of the 12 mutants isolated after this screen, 7 proved to have nonspecific lesions in carbon metabolism. However, five of the mutants, which appeared to result from Tn5 insertions in one or more adjacent loci, were specifically defective in utilizing oleic acid as sole carbon source. Surprisingly, these mutant strains were able to incorporate exogenous fatty acids into lipid, and all of the β-oxidation enzymes were present and had activity comparable to that of the wild type (Table 4).

This failure to isolate mutants with clear-cut lesions in the pathway for fatty acid degradation is in contrast with the situation in E. coli, in which such mutants were readily obtained (20, 25). We were unable to determine the primary lesion which led to the apparent fatty acid degradation-negative phenotype of the mutants described here. However, we encountered a similar situation in C. crescentus during an extensive search for mutants defective in fatty acid biosynthesis (11–13, 16). In that case, none of the fatty acid auxotrophs isolated proved to have mutations in genes encoding fatty acid biosynthetic function. However, several of those mutants had cell cycle phenotypes which suggested a link between lipid metabolism and cell cycle functions. Our inability to obtain mutants in the enzyme of fatty acid degradation, coupled with the high constitutive levels of the fatty acid degradation enzymes, suggests that fatty acid turnover may have additional roles related to the coordination of membrane lipid metabolism and the expression of cell cycle events observed previously in C. crescentus (7, 11–13,

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**TABLE 2.** Effect of glucose on β-oxidation enzyme activities in extracts of C. crescentus and E. coli

| Strain          | Carbon source       | Sp act (nmol/min per mg of protein) of: | Acyl-CoA dehydrogenase |
|-----------------|---------------------|----------------------------------------|------------------------|
|                 |                     | Crotonase | Thiolase | β-HOAH |                        |
| C. crescentus   | Oleic acid          | 2,100     | 790      | 145   | 43                      |
| SC451           | Glucose + oleic acid| 670       | 360      | 69    | 20                      |
| C. crescentus   | Oleic acid          | 3,225     | 345      | 116   | 4                       |
| CB13            | Glucose + oleic acid| 2,576     | 236      | 46    | 3                       |
| Glucose         | 3,106               | 260       | 28       | 3     | 3                       |
| E. coli         | Oleic acid          | 392       | 25       | 37    | 6                       |
| Glucose + oleic acid | 7          | 0.1       | 0.1      | 3     | 3                       |

* C. crescentus SC451 and CB13 and E. coli Le392 were grown in minimal medium supplemented with the indicated carbon sources as described in footnotes a to c of Table 2.

* β-HOAH, β-Hydroxyacyl-CoA dehydrogenase.

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**TABLE 3.** Effect of glucose on β-oxidation enzyme activities in extracts of C. crescentus and E. coli

| Strain          | Carbon source | β-HOAH | Acyl-CoA dehydrogenase |
|-----------------|---------------|--------|------------------------|
|                 |               | 1-HOADH |                        |
| SC451 (CB15)    | 2.79          | 1,880   | 551                    |
| AE4000          | 2.80          | 1,700   | 388                    |
| AE4002          | 2.51          | 1,800   | 423                    |
| AE4010          | 2.14          | 1,123   | 297                    |
| AE4011          | 2.33          | 1,129   | 422                    |
| AE4016          | 2.45          | 1,557   | 452                    |

* β-HOAH, β-Hydroxyacyl-CoA dehydrogenase.
24). We are in the process of generating conditional mutants in fatty acid degradation to test this possibility.

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LITERATURE CITED

1. Binstock, J. F., A. Pramanik, and H. Schulz. 1972. Isolation of a multienzyme complex of fatty acid oxidation from Escherichia coli. Proc. Natl. Acad. Sci. USA 74:492–495.
2. Binstock, J. F., and H. Schulz. 1981. Fatty acid oxidation complex from Escherichia coli. Methods Enzymol. 71:403–411.
3. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911–917.
4. Chow, T. C., and J. M. Schmidt. 1974. Fatty acid composition of Caulobacter crescentus. J. Gen. Microbiol. 83:369–373.
5. Contreras, I., R. Bender, J. Mansour, S. Henry, and L. Shapiro. 1979. Caulobacter crescentus mutant defective in membrane phospholipid synthesis. J. Bacteriol. 140:612–619.
6. Contreras, I., L. Shapiro, and S. Henry. 1978. Membrane phospholipid composition of Caulobacter crescentus. J. Bacteriol. 135:1130–1136.
7. Contreras, I., A. Weisborn, K. Amemiya, J. Mansour, S. Henry, L. Shapiro, and R. Bender. 1980. The effect of termination of membrane phospholipid synthesis on cell cycle dependent events in Caulobacter. J. Mol. Biol. 138:401–409.
8. Ely, B., and R. H. Crot. 1982. Transposon mutagenesis in Caulobacter crescentus. J. Bacteriol. 149:620–625.
9. Feigenbaum, J., and H. Schulz. 1975. Thiolases of Escherichia coli: purification and chain length specificities. J. Bacteriol. 122:407–411.
10. Hall, C. L. 1981. Acyl-CoA dehydrogenases from pig liver mitochondria. Methods Enzymol. 71:375–385.
11. Hodgson, D. A., P. Shaw, V. Letts, S. Henry, and L. Shapiro. 1984. Genetic analysis and characterization of a Caulobacter crescentus mutant defective in membrane biogenesis. J. Bacteriol. 158:430–440.
12. Hodgson, D. A., P. Shaw, M. O’Connell, S. Henry, and L. Shapiro. 1984. Caulobacter crescentus fatty acid-dependent cell cycle mutant. J. Bacteriol. 158:156–162.
13. Hodgson, D. A., P. Shaw, and L. Shapiro. 1984. Isolation and genetic analysis of Caulobacter mutants defective in cell shape and membrane lipid synthesis. Genetics 108:809–826.
14. Klein, K., R. Steinberg, B. Fiethen, and P. Overath. 1971. Fatty acid degradation in Escherichia coli. Eur. J. Biochem. 19:442–450.
15. Komeda, K., and W. D. Nunn. 1981. Purification and characterization of acyl coenzyme A synthetase from Escherichia coli. J. Biol. Chem. 256:5702–5707.
16. Letts, V., P. Shaw, L. Shapiro, and S. Henry. 1982. Synthesis and utilization of fatty acids by wild-type and fatty acid auxotrophs of Caulobacter crescentus. J. Bacteriol. 151:1269–1278.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265–275.
18. Maloy, S. R., M. Bohlander, and W. D. Nunn. 1980. Elevated levels of glyoxylate shunt enzymes in Escherichia coli strains constitutive for fatty acid degradation. J. Bacteriol. 143:720–725.
19. Moncla, B. J., S. L. Hillier, and W. T. Charnetzky. 1983. Constitutive uptake and degradation of fatty acids by Yersinia pestis. J. Bacteriol. 153:340–344.
20. Overath, P., G. Pauli, and H. U. Schairer. 1969. Fatty acid degradation in Escherichia coli. Eur. J. Biochem. 7:559–745.
21. Pauli, G., R. Ehring, and P. Overath. 1974. Fatty acid degradation in Escherichia coli: requirement of cyclic adenosine monophosphate and cyclic adenosine monophosphate receptor protein for enzyme synthesis. J. Bacteriol. 117:1178–1183.
22. Pindexter, J. S. 1964. Biological properties and classification of the Caulobacter group. Bacteriol. Rev. 28:231–295.
23. Shapiro, L. 1985. Generation of polarity during Caulobacter cell differentiation. Annu. Rev. Cell Biol. 1:225–259.
24. Shapiro, L., J. Mansour, P. Shaw, and S. Henry. 1982. Synthesis of specific membrane proteins is a function of DNA replication and phospholipid synthesis in Caulobacter crescentus. J. Mol. Biol. 159:303–322.
25. Simons, R. W., P. A. Egger, H. T. Chute, and W. D. Nunn. 1980. Regulation of fatty acid degradation in Escherichia coli: isolation and characterization of strains bearing insertion and temperature-sensitive mutations in gene fadR. J. Bacteriol. 142:621–632.
26. Spratt, S. K., P. N. Black, M. M. Ragozzino, and W. D. Nunn. 1984. Cloning, mapping, and expression of genes involved in the fatty acid-degradative multienzyme complex of Escherichia coli. J. Bacteriol. 158:535–542.
27. Weeks, G., M. Shapiro, R. O. Burns, and S. J. Wakil. 1969. Control of fatty acid metabolism. 1. Induction of the enzymes of fatty acid oxidation in Escherichia coli. J. Bacteriol. 97:827–836.