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The Putative *fabJ* Gene of *Escherichia coli* Fatty Acid Synthesis Is the *fabF* Gene

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Siggaard-Andersen and coworkers (M. Siggaard-Andersen, M. Wissenbach, J. Chuck, I. Svendsen, J. G. Olsen, and P. von Wettstein-Knowles, Proc. Natl. Acad. Sci. USA 91:11027–11031, 1994) recently reported the DNA sequence of a gene encoding a β-ketoacyl–acyl carrier protein synthase from *Escherichia coli*. These workers assigned this gene the designation *fabJ* and reported that the gene encoded a new β-ketoacyl–acyl carrier protein synthase. We report that the *fabJ* gene is the previously reported *fabF* gene that encodes the known β-ketoacyl–acyl carrier protein synthase II.

Three β-ketoacyl–acyl carrier protein (ACP) synthase (KAS) enzymes that catalyze the condensation-chain elongation reaction of fatty acid synthesis have been demonstrated in *Escherichia coli*. Each of the three enzymes (called KAS I, KAS II, and KAS III) has been purified to homogeneity, and the differing substrate specificities of the enzymes have been studied by direct assay with defined substrates (3, 4, 14). Moreover, the *fabB* and *fabH* genes have been demonstrated to encode KAS I and KAS III, respectively, by standard genetic analyses (3, 4, 14) plus overproduction of the enzymes from plasmid clones and correspondence of the determined amino termini with those deduced from DNA sequence data (7, 14). As noted previously (10), more detailed study of KAS II has been hindered by the extreme instability of the clones carrying the intact *fabF* gene. The *fabF* gene was assigned as the KAS II structural gene, since *fabF* mutants specifically lack this activity (3, 15). The *fabF* mutations were mapped very close to the gene (*fabD*) encoding malonyl-coenzyme A-ACP transacylase, the enzyme responsible for synthesis of malonyl-ACP, a substrate of the KAS enzymes (15). More recent work has shown that *E. coli* contains a cluster of fatty acid synthetic genes which are transcribed clockwise on the genetic map in the order *fabH*, *fabD*, *fabG*, and *acpP* and which encode KAS III, malonyl-coenzyme A-ACP transacylase, β-ketoacyl–ACP reductase, and ACP, respectively (10, 11). The sequence located downstream of *acpP* was assigned to *fabF* (or a gene whose expression was polar on *fabF*) because insertion of a DNA segment encoding kanamycin resistance (Kan^r) into an *Nru*I site 570 bp downstream of *acpP* resulted in a *fabF* phenotype (11). As noted previously (9), we had sequenced cloned chromosomal segments which together with several PCR products (amplified directly from the *E. coli* chromosome) gave a complete open reading frame (ORF) of 1,239 bp with a deduced amino acid sequence 37.6% identical to that of the *fabB* gene encoding KAS I (8). The ORF ended just 5′ to the gene encoding the *pabC* gene of *p*-aminobenzoate synthesis (6). However, we delayed publication of these data until we could understand (and overcome) the instability of *fabF* clones and overproduce, purify, and characterize the enzyme to obtain direct evidence that the ORF does indeed encode KAS II.

Recently, Siggaard-Andersen et al. (13) also sequenced the *E. coli* chromosomal region just downstream of *acpP* and obtained a sequence identical to that which we had obtained. These workers assigned this sequence to a putative new KAS called KAS IV rather than to KAS II. Their assignment is based on the isolation of proteins covalently labeled with ^{3}H-cerulenin, an inhibitor of most KAS enzymes (including KAS II), together with the partial fractionation of crude cell extracts and the restoration of fatty acid synthesis to cerulenin-treated extracts (13). On the basis of these data, they reported that *E. coli* contains a fourth KAS activity (KAS IV) specific for the synthesis of short-chain (C_4, C_6, and C_8) acids.

We report that the KAS enzyme coded for just downstream of *acpP* is KAS II (FabF) and not a new KAS. This conclusion is based on the following data. First, the *Nru*I insertion noted above lies in the N-terminal half of the ORF just upstream of the putative active-site serine (an alignment of KAS II with KAS I is given in reference 13). Strains in which this insertion was recombined into the chromosome had a *fabF* phenotype as shown by fatty acid compositional data (Table 1) and the lack of a protein band cross-linked to ACP previously assigned to KAS II (Fig. 1). (These data were mentioned but were not included in the original report [11] because of space limitations.) The *fabF1* mutant was first recognized by its decreased *cis*-vaccenate (18:1) and increased palmitoleate (16:1) content and the insensitivity of the *cis*-vaccenate (18:1) content to growth temperature (5). The insertion mutant has a similar fatty acid compositional phenotype (Table 1). Moreover, introduction of a temperature-sensitive *fabB* lesion into the insertion mutant resulted in the loss of the ability to grow at 42°C on oleic acid-supplemented media (11). This is the established phenotype of *fabB fabF* double mutant strains (3), indicating that KAS I and KAS II are the major enzymes involved in the synthesis of long-chain saturated fatty acids. Second, transductional crosses between the Kan^r insertion strain and a *fabB15 fabF1* strain gave no *fabF*$^+$ recombinants. Third, introduction of plasmid pKM44 carrying the *Nru*I-*Bgl*II fragment containing the 3′ 60% of the wild-type gene (the *Bgl*II site overlaps the *fabF* termination codon) into *fabB15 fabF1* and *fabB15 fabF3* strains resulted in rescue of growth at 42°C in the presence of oleate. Growth was due to the recombinational marker rescue of the *fabF* mutation, since colony formation was dependent on homologous recombination (rescue was blocked by introduc-

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TABLE 1. Fatty acid compositions of the membrane phospholipids

Strain[a]	Growth temp (°C)	Weight (%) of fatty acid ester[b]		
		16:0	16:1	18:1
SJ16	30	36.4	31.0	28.1
SJ16	42	45.6	31.6	17.6
MR86	30	27.7	56.8	6.4
MR86	42	40.3	47.0	5.1

[a] Strain SJ16 (*panD2 gyrA216 metB1 relA spoT zad-220*::Tn*10*) is wild type in fatty acid composition, whereas strain MR86 was derived from SJ16 by transduction with P1*vir* phage grown on a derivative of strain JC7623 into which the *fabF*::Kan^r insertion (constructed by insertion of the *Hin*cII fragment of pUC4K from Pharmacia into the *fabF Nru*I site) had been recombined into the chromosome (16). The strains were grown in a medium of 1% tryptone, 0.1% yeast extract, and 0.5% NaCl and harvested at mid-log phase. The phospholipids were extracted, and their fatty acid moieties were converted to methyl esters in sodium methoxide-methanol and then analyzed by gas chromatography on a column (15 m by 0.53 mm) of 50% cyanopropyl polysiloxane (DB23; J & W Scientific) ramped from 130 to 170°C at 2.5°C/min followed by a 5°C/min ramp to 230°C.

[b] The fatty acids given are 16:0, palmitic acid; 16:1, palmitoleic acid (including its cyclopropane derivative); and 18:1, *cis*-vaccenic acid (including its cyclopropane derivative). Minor amounts of myristic and stearic acids were also present.

tion of a *recA* mutation into the *fabB fabF* strains) and the recombinants retained a requirement for oleic acid at 42°C (the *fabB15* phenotype) (3, 15). Fourth, sequence analysis of PCR products derived from the chromosomal DNAs of *fabF1* and *fabF3* strains gave evidence for mutations within the C-terminal region of the ORF. Both *fabF* strains contained two G-to-A transitions, one of which converted Ser-220 to Asn whereas the other converted Gly-262 to Met.

From these data, it seems clear that the sequence reported by Siggaard-Andersen et al. (13) is that of the *fabF* gene. Genetic nomenclature is based on the phenotypes of mutants, and this DNA segment clearly is the locus of the original *fabF* mutation. Moreover, disruption of the ORF gave a *fabF* phenotype. Thus, the gene must be designated *fabF*, not *fabJ*. In our opinion, the presence of a fourth *E. coli* KAS remains an open question. Our most straightforward interpretation of the data reported by Siggaard-Andersen et al. (13) is that the enzyme preparation studied may consist of KAS III mixed with smaller amounts of KAS II (and/or KAS I). KAS III has a substrate specificity very similar to that reported for KAS IV by Siggaard-Andersen et al. (13) and is insensitive to cerulenin (14). Since cerulenin labeling was a primary means used by Siggaard-Andersen and coworkers (13) to detect KAS proteins, KAS III may have escaped detection by these workers (no elution position for KAS III was designated in the fractionation profile of crude *E. coli* extracts reported by these workers). KAS III mixed with lesser amounts of the cerulenin-sensitive KAS enzymes could account for both the substrate specificity and the partial cerulenin sensitivity reported. It should be noted that the correspondence between the putative KAS IV and the protein band subjected to amino acid sequencing was indirect. Siggaard-Andersen et al. (13) could only "infer the KAS activity was associated with the latter protein." However, the finding that this N-terminal sequence matched that deduced from the *fabF* DNA sequence did document the use of the atypical GUG codon in the initiation of *fabF* translation.

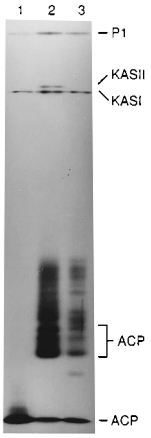

FIG. 1. β-[3-^{3}H]alanine-labeled proteins. The fluorogram of a native gel electrophoretic separation of the proteins of β-[3-^{3}H]alanine-labeled cells is shown. The labeling of the KAS proteins is indirect (12); a noncovalent KAS-ACP complex is converted to a covalent KAS-ACP disulfide-linked species by the loss of the cytosolic-reducing environment that results upon cell lysis (15). The disulfides are formed between the ACP prosthetic group thiol and that of the KAS active-site cysteine residues (12, 15). The gel was run in the absence of a reducing agent so that the proteins cross-linked to ACP (KAS I and KAS II) or coenzyme A (P1) in addition to the proteins covalently labeled with β-[3-^{3}H]alanine could be visualized (12). The experiment was done and the proteins were identified as described previously (15). All strains carried a *panD* mutation. Lane 1, strain MR97 (*fabF1*); lane 2, strain SJ16 (wild type); lane 3, strain MR86 (*fabF*::Kan^r). The lack of more slowly migrating ACP forms in lane 1 is due to the faster-migrating form of ACP associated with *fabF1* mutants (7).

Several other aspects of the KAS enzymes deserve mention. First, it should be noted that *fabF*::Kan^r insertion strains are less defective in *cis*-vaccenic acid biosynthesis than are the original (double) point mutants (Table 1). Since the insertion into *fabF* is very close to the putative active-site cysteine and disrupts the conserved active-site sequence, it seems unlikely that the disruption mutant retains partial activity. Moreover, the *fabF*::Kan^r insertion results in the loss of detectable *fabF* mRNA in Northern (RNA) hybridization experiments (17). Therefore, it seems more likely that the mutant FabF proteins inhibit the low level of *cis*-vaccenic acid synthesis catalyzed by KAS I (FabB) (10), perhaps by forming heterodimers with FabB monomers or by blocking interaction of FabB with other fatty acid biosynthetic enzymes. This is consistent with the partial dominance of the *fabF1* allele over the wild-type allele (15). This notion might also account for the surprising finding that two independently derived *fabF* mutants contain the same pair of amino acid changes. It is interesting that both mutations lie in regions where the sequences of FabF and FabB are identical. An intriguing possibility is that the postulated KAS enzyme heterodimers might have substrate specificities that differ from those of the homodimeric proteins. Second, the mutational lesion in Vtr strains that overproduce *cis*-vaccenic acid at all growth temperatures (1, 2) maps within or very close to the *fab* cluster (15). The most straightforward interpretation of these data was that the Vtr lesion is a *fabF* allele that results in higher levels of KAS II activity. However, previous workers in our laboratory were unable to detect any changes in KAS II activity or substrate affinity in preparations from Vtr strains (15). In the course of the present work, we amplified *fabF* gene DNA segments from the chromosome of a Vtr strain by PCR and found that the *fabF* sequence was identical to that of the wild-type gene. It therefore seems that the Vtr mutation affects another gene of the *fab* cluster. This notion is consistent with the partial genetic dominance of the Vtr lesion over the wild-type and *fabF1* alleles (15). Finally, it should be noted that neither we nor Siggaard-Andersen and coworkers (13) have cloned the *fabF* gene in a form demonstrated to encode the active enzyme.

Nucleotide sequence accession number. The sequence reported on in this work has been assigned GenBank accession number U20767.

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