JOURNAL OF BACTERIOLOGY, Sept. 1996, p. 5382–5387 Vol. 178, No. 18
0021-9193/96/$04.00+0


# Increased Unsaturated Fatty Acid Production Associated with a Suppressor of the *fabA6*(Ts) Mutation in *Escherichia coli*

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Received 19 October 1995/Accepted 11 July 1996

**Plasmids that corrected the temperature-sensitive unsaturated fatty acid auxotrophy of strain M6 [*fabA6* (Ts)] were isolated from an *Escherichia coli* genomic library. Subcloning and physical mapping localized the new gene (called *sfa* for suppressor of *fabA*) at 1,070 kb on the *E. coli* chromosome. DNA sequencing revealed the presence of a 227-bp open reading frame which directed the synthesis of a peptide of approximately 8 kDa, which correlated with the correction of the *fabA6*(Ts) phenotype. However, the *sfa* gene was an allele-specific suppressor since plasmids harboring the *sfa* gene corrected the growth phenotype of *fabA6*(Ts) mutants but did not correct the growth of *fabA2*(Ts) or *fabB15*(Ts) unsaturated fatty acid auxotrophs. Overexpression of the *sfa* gene in *fabA6*(Ts) mutants restored unsaturated fatty acid content at 42°C, and overexpression in wild-type cells resulted in a substantial increase in the unsaturated fatty acid content of the membrane. Thus, the suppression of the *fabA6*(Ts) mutation by *sfa* was attributed to its ability to increase the biosynthesis of unsaturated fatty acids.**

Unsaturated fatty acids are absolutely required for the normal growth of *Escherichia coli* (9), and the investigation of the enzymes responsible for the formation of these fatty acids and their regulation remains an area of active research (21). In *E. coli*, there are two gene products known to be required for unsaturated fatty acid synthesis. The product of the *fabA* gene, β-hydroxydecanoyl-acyl carrier protein (ACP) dehydratase, catalyzes the dehydration of β-hydroxydecanoyl-ACP to a mixture of *trans*-2-decenoyl-ACP and *cis*-3-decenoyl-ACP, thus introducing the *cis* double bond into the growing fatty acid chain. The *trans*-2 isomer is the normal intermediate in saturated fatty acid synthesis and is converted to saturated fatty acids following reduction of the double bond by enoyl-ACP reductase (*fabI*) (14). The double bond in the *cis*-3 intermediate is preserved, and the 10-carbon intermediate is elongated to form the unsaturated fatty acids. The essential nature of FabA is clear from the isolation of mutants defective in this enzyme activity (26). These mutants cannot make unsaturated fatty acids, although saturated fatty acid synthesis is not affected. The nucleotide sequence of the *fabA* gene is known (10), and the regulation of *fabA* expression involves the transcriptional activator, FadR, which also functions as a repressor of fatty acid β-oxidation genes (15, 16). The first indication of a second enzyme required for unsaturated fatty acid biosynthesis was that the unsaturated fatty acid auxotrophs could be divided into two complementation groups (8). The *fabA* gene maps to minute 21.9 on the *E. coli* chromosome (11), whereas the second mutation, termed *fabB*, maps to minute 52.6 (5). The second essential enzyme is β-ketoacyl-ACP synthase I, the product of the *fabB* gene (24). This condensing enzyme carries out an essential step in unsaturated fatty acid synthesis, which is most likely the elongation of *cis*-3-decenoyl-ACP. The *fabB* gene has been cloned (12) and sequenced (18); however, specific transcriptional regulators, if any, have not been identified.

The ratio of saturated to unsaturated fatty acids is controlled by the relative levels of FabA and FabB. Overproduction of FabA leads to an increased synthesis of saturated fatty acids (6). Thus, the *fabA* gene product is essential for unsaturated fatty acid synthesis, although the level of enzyme is not a rate-limiting factor in determining the proportion of unsaturated fatty acids produced by the pathway. The overexpression of the *fabB* gene corrects the effect of *fabA* overexpression on the accumulation of saturated fatty acids (6) and leads to an increase in unsaturated fatty acid content in normal strains (12). These data argue that the level of FabB expression relative to that of FabA is an important factor in specifying the saturated/unsaturated fatty acid ratio in membrane phospholipids (21). In this paper, we identify a genetic locus that modifies this regulatory network that was isolated by screening a genomic library for second-site suppressors of *fabA* mutations. This report describes the isolation of a gene termed *sfa* (for suppressor of *fabA*) that suppresses the *fabA6*(Ts) temperature-sensitive growth phenotype. Overexpression of the *sfa* gene leads to the overproduction of unsaturated fatty acids in wild-type strains.

## MATERIALS AND METHODS

**Bacterial strains.** All bacterial strains were derivatives of *E. coli* K-12 and are listed in Table 1. The permissive growth temperature for *fabA*(Ts) mutants was 30°C, and the nonpermissive growth temperature was 42°C. Rich medium was composed of 10 g of tryptone per liter, 5 g of NaCl per liter, and 1 g of yeast extract per liter, and minimal medium E and M9 medium were formulated as described by Miller (23). Minimal medium was prepared by supplementing the salt solution with glucose (0.4%), thiamine (0.0005%), and required amino acids (0.01%).

**Isolation of the *sfa* clone.** The *E. coli* genomic library used to isolate *sfa* was constructed by C. DiRusso (13, 17). *E. coli* chromosomal DNA was partially digested with *Sau*3A and size selected for fragments between 5 and 20 kb. DNA was ligated into the *Bam*HI site of pBR322, and the ligation mix was transformed into strain LE392. Plasmids derived from a pool of about 5,000 $Amp^r$ colonies were isolated and used as the library. Strain M6 [*fabA6*(Ts)] was transformed with the library and selected on rich plates containing ampicillin at 42°C. Plasmids were isolated from each of the transformants, and strain M6 was transformed a second time with each of the candidate plasmids to screen out revertants. Plasmids were isolated from each of the positive colonies and screened by

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TABLE 1. Bacterial strains

| Strain | Genotype | Source or reference |
|---|---|---|
| C600 | *leuB6 thr-1 lacY1 thi-1 supE44 tonA33* $\lambda^-$ $F^-$ | CGSC[a] |
| CY50 | *fabA2*(Ts) *trp his thi-1 gal xyl mt1* | J. Cronan |
| CY274 | *fabB15*(Ts) | J. Cronan |
| JT2600 | *fabA6*(Ts) *zcf*::Tn*10* | P1(DC303) × M6[b] |
| JT2602 | *fabA6*(Ts) *zdf*::Tn*10 leuB6 thr-1 lacY1 thi-1 supE44 tonA33* $\lambda^-$ $F^-$ | P1(JT2600) × C600[b] |
| DC303 | *zcf*::Tn*10 trp his thi-1 gal xyl mtl* | D. Clark |
| M6 | *fabA6*(Ts) *supD* $F^-$ | 4 |

[a] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.
[b] Transductions were performed with P1*vir* bacteriophage; the donor strain is shown in parentheses, and the selections were for $Tet^r$.

digestion with *Nco*I. Plasmid pJTJ1 was selected for further study because it lacked the 719-bp *Nco*I fragment characteristic of the *fabA* gene (10). Plasmid DNA isolation, restriction enzyme digestions, and agarose slab gel electrophoresis were performed as described previously (22).

Subclones of the original pJTJ1 plasmid were constructed by standard molecular biology techniques (25). Plasmids were constructed by the extraction of DNA fragments following agarose gel electrophoresis followed by ligation into plasmid pBR322 that had been digested with the appropriate restriction enzymes. Digestion of plasmid pJTJ1 with *Eco*RI yielded fragments of 4.5, 3.9, and 3.3 kb. The 3.9-kb *Eco*RI fragment was cloned into pBR322 to yield pJTJ3, and the 3.3-kb fragment was used to generate plasmid pTJT4. Plasmid pJTJ5 was derived from the 1.6-kb *Bam*HI fragment from pJTJ4, plasmid pJTJ6 was derived from an *Eco*RI-*Eco*RV fragment of pJTJ4, plasmid pJTJ7 was derived from a *Bam*HI-*Eco*RV fragment of pJTJ4, and plasmid pJTJ8 was derived from an *Eco*RI-*Bam*HI fragment of pJTJ4. Plasmid pSJ139 was constructed by PCR amplification of pJTJ8 with a primer (SF1) that annealed to bases 218 to 241 containing an engineered *Sph*I site (5′-CGACGCATGCTCACCTTGATAAG-3′) and a second primer (SR1) that annealed to bases 664 to 690 containing an engineered *Sal*I site (5′-CATCACGTCGACGGGGTGATGTTCAC-3′). This fragment containing the *sfa* gene was cloned into pBS and designated pSJ139.

**Physical mapping of *sfa*.** The *E. coli* gene mapping membrane (Takara Shuza Co., Ltd.) containing the Kohara miniset of lambda phage DNA was hybridized with a $^{32}P$-labeled probe derived from the 1.6-kb *Bam*HI fragment of pJTJ1 as described in the directions of the manufacturer. Hybridizing phages were detected by autoradiography and identified by comparing the autoradiograph with the key provided by the manufacturer. The location of the insert in pJTJ1 was then determined by aligning the restriction map with the genomic restriction map (19).

**DNA sequencing.** Plasmid DNA for sequencing was routinely isolated from overnight cultures with either a Stratagene plasmid-quick or Qiagen plasmid purification kit. Subclones of the *Bam*HI-*Eco*RI insert in plasmid pJTJ8 were generated with the *Kpn*I, *Bsm*I, *Pfl*MI, and *Hin*cII restriction enzymes, and these subclones were used to sequence both strands of the insert with the M13 universal primers and the automated DNA sequencing instrumentation (Applied Biosystems, Inc.) provided by the St. Jude Molecular Resource Center.

The *fabA2* and *fabA6* alleles were sequenced following PCR amplification and the cloning of two fragments corresponding to half of each of the genes from chromosomal DNA derived from strains CY50 [*fabA2*(Ts)] and M6 [*fabA6*(Ts)], respectively. The 5′ halves of the *fabA* alleles were amplified with primer RB1A (5′-ACGTTGGCTGAATTCGTTTATTCC-3′) containing an engineered *Eco*RI site complementary to the sequence just downstream of the *Nco*I site and primer RB1B (5′-TATCAAGCTTAGCCGAGGTAGAACCCTAC-3′) containing an engineered *Hin*dIII site complementary to a region just downstream of the *Pvu*II site located approximately in the middle of the *fabA* gene. The 3′ half of the gene was amplified with primer RB2B (5′-TATCAAGCTTGGCCTGGACGCAATG TG-3′) complementary to a region 5′ of the *Pvu*II site containing an engineered *Hin*dIII site and primer RB2A (5′-ATTGCGGAGGATCCGCCTTTTG-3′) complementary to the sequence downstream of the stop codon and containing an engineered *Bam*HI site. The PCR products were cloned into pBluescript KS, and both strands of four independent clones were sequenced with the universal M13 primers and automated sequencing instrumentation (Applied Biosystems) provided by the St. Jude Molecular Resource Center.

**Protein and RNA expression.** The proteins expressed by each of the subclones of plasmid pJTJ1 were analyzed with the transcription-translation kit supplied by Promega. The proteins were labeled with [$^{35}S$]methionine (New England Nuclear Corp.; specific activity, 1,000 Ci/mmol) and separated by sodium dodecyl sulfate-gel electrophoresis on a 15% polyacrylamide gel. Labeled protein bands were visualized by autoradiography. Total RNA from strain C600 was isolated (1), and the *sfa* mRNA was detected by Northern (RNA) blot analysis (27) with the 500-bp *Bsm*I-*Bam*HI fragment derived from plasmid pJTJ8.

**Purification of the FabA6 protein.** The FabA6 protein was purified by PCR amplification of the *fabA6* gene from strain M6, cloning the product into the PET-15b His-tag expression vector and purification of the protein with $Ni^{2+}$ affinity chromatography as described for the normal FabA protein (14). The activity of the protein was determined in assays containing the six enzymes required to carry out the first cycle of fatty acid elongation, and the products were separated by conformationally sensitive gel electrophoresis and visualized by fluorography (14).

**Fatty acid composition.** The fatty acid composition of *E. coli* strains was determined essentially as described previously (7). Cells were grown to a density of $5 \times 10^8$ cells per ml in rich broth, the cells were harvested, and the lipids were extracted (3). Fatty acid methyl esters were prepared by reacting the lipid sample with 5% HCl in anhydrous methanol. The fatty acid compositions were determined with a HP5890 gas chromatograph equipped with a column packed with Supelcoport (100/120 mesh) coated with 5% DEGS-PS and operated isothermally at 165°C.

**Nucleotide sequence accession number.** The nucleotide sequences described in this paper were submitted to GenBank under the accession number U38541 for the *sfa* gene and adjacent DNA, accession number U37057 for the *fabA6*(Ts) allele, and accession number U56977 for the *fabA2*(Ts) allele.

## RESULTS AND DISCUSSION

**Isolation of the *sfa* clone.** Strain M6 [*fabA6*(Ts)] was transformed with a chromosomal library, and recombinant plasmids that corrected the temperature-sensitive growth phenotype were isolated at 42°C. Plasmid isolation and retransformation confirmed that the phenotype correction was due to the presence of the plasmid and not to reversion. The remaining candidate plasmids were digested with *Nco*I and screened for a 719-bp fragment which was diagnostic for the *fabA* gene. The plasmids possessing the 719-bp *Nco*I fragment were not studied further. Restriction enzyme mapping of the remaining candidate plasmid, designated pJTJ1, verified that it did not contain the *fabA* locus.

We transformed two other temperature-sensitive unsaturated fatty acid auxotrophs with the pJTJ1 plasmid to determine the spectrum of mutants that this plasmid could complement. Plasmid pJTJ1 did not permit the growth of either strain CY274 [*fabB15*(Ts)] or strain CY50 [*fabA2*(Ts)] at 42°C. We also transferred the *fabA6*(Ts) allele into the strain C600 genetic background by first introducing the *zcf*::Tn*10* from strain DC303 into strain M6 and then moving the transposon plus the *fabA6*(Ts) allele into strain C600 by P1*vir*-mediated transduction. The resulting strain was a temperature-sensitive unsaturated fatty acid auxotroph, and its ability to grow at 42°C in the absence of oleate was restored by the presence of plasmid pJTJ1. These results support the idea that plasmid pJTJ1 expressed an allele-specific suppressor of the temperature-sensitive *fabA6*(Ts) unsaturated fatty acid auxotroph phenotype.

**Location of the *sfa* gene on the *E. coli* chromosome.** The origin of the chromosomal DNA insert in pJTJ1 was determined by hybridizing the $^{32}P$-labeled 1.6-kb *Bam*HI fragment to a panel of overlapping lambda phage clones derived from the Kohara library dot-blotted onto a hybridization membrane. Only two lambda phage clones in the miniset (λ225 and λ226) hybridized with the labeled probe. A comparison of the restriction enzyme map of pJTJ1 with the map of the region of the chromosome between 1,060 and 1,080 kb is shown in Fig. 1. The *fabA* gene is located between 1,028 and 1,030 kb on the physical map.

The insert in plasmid pJTJ1 was subcloned, and each of these smaller clones was tested for its ability to suppress the *fabA6*(Ts) growth phenotype of strain M6. The results from this series of complementation experiments are summarized in Fig. 2 and clearly localized the *sfa* gene within the 1.0-kb *Eco*RI-*Bam*HI fragment of plasmid pJTJ1. Like pJTJ1, the plasmid containing the 1.0-kb *Eco*RI-*Bam*HI fragment (pJTJ8) did not complement the growth of strain CY50 [*fabA2*(Ts)].

**Correlation of protein expression with phenotypic suppression.** The number and approximate molecular size of the pro-

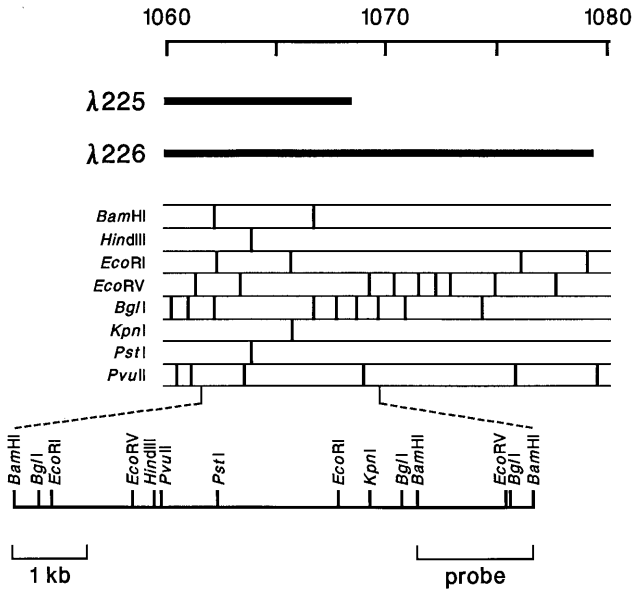


FIG. 1. Location of the DNA insert in plasmid pJTJ1 on the *E. coli* chromosome. A $^{32}$P-labeled probe was synthesized from the 1.5-kb *Bam*HI fragment of the insert DNA and hybridized to the *E. coli* gene mapping membrane. The probe hybridized to λ225 and λ226, and the restriction map of pJTJ1 was aligned with the restriction map of the chromosome in the region of overlap between the two phages.

teins expressed by each of the subclones in Fig. 2 were analyzed with a bacterial transcription-translation system (Fig. 3). All of the clones expressed the β-lactamase protein (Bla), and several other higher- and lower-molecular-weight proteins were detected in control incubations with plasmid pBR322 (Fig. 3). A low-molecular-weight protein (approximately 8 kDa) was detected in each plasmid capable of suppressing the *fabA6*(Ts) phenotype and was not expressed from plasmids that did not reverse the temperature-sensitive growth of strain M6 (compare Fig. 2 and 3). The 8-kDa protein was the only protein significantly expressed from plasmid pJTJ8 compared with the control. These data suggest that the expression of the low-molecular-weight protein is responsible for the allele-specific suppression activity of plasmid pJTJ8.

A $^{32}$P-labeled probe was synthesized from the *Bsm*AI-*Bam*HI fragment derived from plasmid pJTJ8 and was used to detect *sfa* mRNA by Northern blot analysis of total *E. coli* mRNA. This probe hybridized with a band of approximately 350 bp, indicating that the *sfa* gene was expressed in growing cells. The intensity of the putative *sfa* band compared with the intensity of the *fabA* mRNA detected in the same experiment as a control indicated that the *sfa* gene was expressed at about 20% of the level of *fabA*.

**Sequence of the *sfa* gene.** The 1.0-kb *Eco*RI-*Bam*HI chromosomal fragment in plasmid pJTJ8 was sequenced (Fig. 4). A 231-bp open reading frame, designated the *sfa* gene and beginning at nucleotide 267 and ending at nucleotide 495, predicted to encode a protein of 77 amino acids with an isoelectric point of pH 6.36 was found located in the sequence of plasmid pJTJ8. Upstream of the *sfa* gene is an inverted repeat sequence (nucleotides 155 to 180) that is predicted to form a hairpin structure ($\Delta G = -18.4$) and is followed by a run of T's that predict a rho-independent terminator. These data suggest that the sequences between nucleotides 1 and 215 are the 3′ end of an upstream transcription unit. The predicted size of the Sfa protein, 8.837 kDa, was consistent with the size of the protein expressed by plasmid pJTJ8 (Fig. 3). We did not find DNA or protein sequences in the nonredundant nucleotide or protein databases that were significantly similar to the *sfa* gene by use of the BLAST programs. We did find a region between nucleotides 489 and 669 of the insert in pJTJ8 that was similar to a sequence found in the ECOS1 region of the chromosome (20); however, the significance of this DNA sequence similarity is unclear.

**Identification of the mutations in the *fabA2*(Ts) and *fabA6* (Ts) alleles.** Our data suggested that *sfa* was an allele-specific suppressor of the *fabA*(Ts) phenotype. This hypothesis predicts that strains CY50 and M6 express different temperature-sensitive FabA enzymes. This point was verified by DNA sequence analysis of the *fabA2* and *fabA6* alleles following PCR amplification of the *fabA* genes from strains CY50 and M6, respectively (see Materials and Methods). These two new sequences were compared with the *fabA* sequence (10). The numbering of the *fabA* nucleotide sequence in this report begins at the ini-

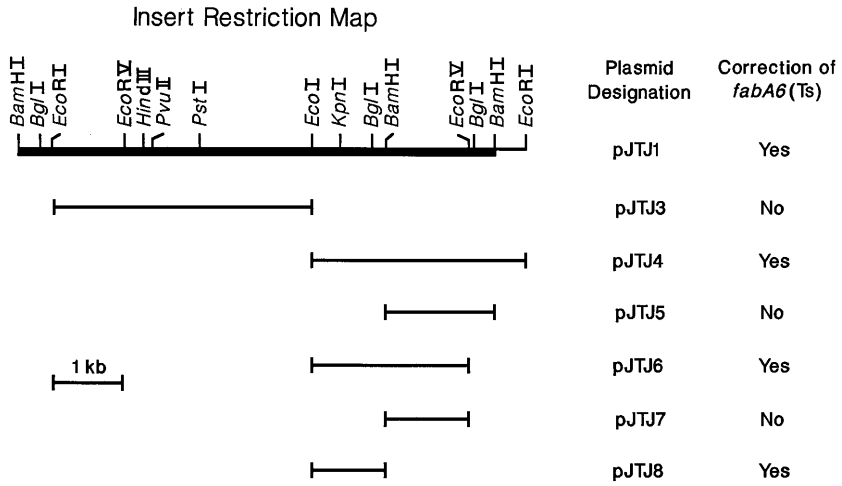


FIG. 2. Localization of the *sfa* gene. Different segments of the parent plasmid pJTJ1 were subcloned into pBR322 with the restriction enzymes indicated, and the individual subclones were tested for their ability to correct the *fabA6*(Ts) growth phenotype.

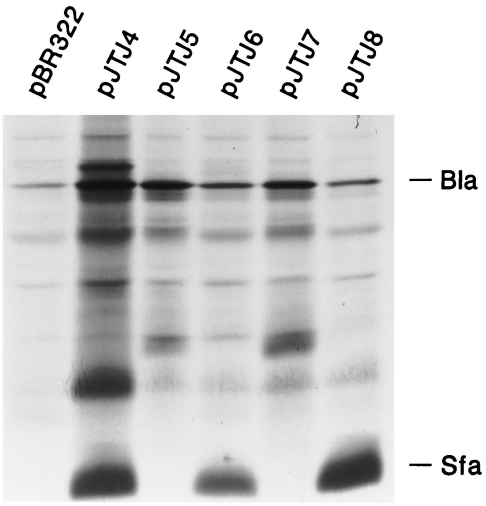


FIG. 3. Transcription-translation analysis. The number and approximate size of the proteins expressed by each of the plasmid subclones tested in Fig. 2 were determined with a transcription-translation system described in Materials and Methods. Each of the plasmids expressed the *bla* gene encoding β-lactamase (Bla). The plasmids capable of suppressing *fabA6*(Ts) all expressed the low-molecular-weight protein designated Sfa.

tiator ATG of the *fabA* gene. Sequence analysis revealed that the *fabA2* allele contained a G-to-A mutation at position 305 (G305-A) of the nucleotide sequence which is predicted to result in the expression of a G102-D FabA2 mutant protein. The *fabA6* allele contained a C102-T mutation which is predicted to give rise to the expression of a P76-L FabA6 mutant protein. These data verify that the temperature-sensitive FabA proteins expressed in strains CY50 [*fabA2*(Ts)] and M6 [*fabA6*(Ts)] represent unique mutations that give rise to a temperature-sensitive unsaturated fatty acid growth phenotype.

In addition to these two mutations, we also found two differences between both of the *fabA* alleles sequenced in this study and the originally reported *fabA* sequence (10). In all clones, a T in place of an A at position 51 of the *fabA* nucleotide sequence was detected; however, this mutation does not result in a change in the amino acid sequence of the protein. The second difference occurred at the carboxy terminus of the protein. The last three residues in the original *fabA* sequence (10) were TLF encoded by the DNA sequence ACT CTG TTC TAG. We found a different DNA sequence in this region as a result of the presence of three additional bases (shown in lowercase letters), yielding the sequence ACg TCT Gcc TTC TGA, which leads to a predicted carboxy-terminal protein sequence of TSAF. Digestion of purified FabA with carboxypeptidase Y released amino acids in the order of abundance F > A > S > T (10). Subsequent analysis of *fabA* sequences from different *E. coli* strains also showed a carboxy-terminal sequence identical to the sequence we found in the *fabA2* and *fabA6* alleles (2). Thus, there appear to be two variants of the *fabA* gene in *E. coli* that differ in their carboxy-terminal sequences.

The defect in the *fabA6* allele was established to be a deficiency in the activity of the protein by cloning the *fabA6* gene by PCR amplification of genomic DNA from strain M6, cloning the DNA into the pET-15b His-tag expression vector, and purifying the protein by affinity chromatography as we previously described for the wild-type *fabA* allele (14). Although several milligrams of protein was isolated, the specific activity of the FabA6 protein was >100-fold less than that of the

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   1 CGAATTCCGCACGCTGAACGAAAATCAGAAAGTTGCAATTTTCTATTGAGCAGGGGCAACGTGGCCCCGC

  71 GGCAGCAACGTTGTTACGCTCTAAGGTTGCCATTATTACTCAACATCTCCATTTCCGCTGTCCATGTTGT

 141 CATGGTTCACAGTACCGCACATCGGCATTCGATGTGACGGAGCGAAACCCTTTGGGCGCTAAGTGTATTT

 211 TTTGTAAATCGACGATGATCACCTTTGATAACGTCGCGCTGCAAATACGCACTGACCATGCGCCGCTGGA
                                                              M  R  R  W

 281 TTTCACAAAATAATATCAGGCTCCCTCGTGGAGCCTTTTTTATATCTGCCTTATTTTTCTTCAACGCTGT
     I  S  Q  N  N  I  R  L  P  R  G  A  F  F  I  S  A  L  F  F  F  N  A  V

 351 ATGTATAGTAAGCGATAACCTGTTGATTATTGAATCTTTCGGGGAGATGGCTTATAACATTTCTTACCTG
       C  I  V  S  D  N  L  L  I  I  E  S  F  G  E  M  A  Y  N  I  S  Y  L

 421 ACCAGGGTACCGGGAACCAACACCTTACTGGCGTGTTGCTGTCTTTTAAGACCAGAAGAGGTTAACAGTG
      T  R  V  P  G  T  N  T  L  L  A  C  C  C  L  L  R  P  E  E  V  N  S

 491 AATATTGAAGAGTTAAAAAAAACAAGCCGAAACGGAAATCGCCGACTTTATCGCGCAAAAAATCGCCGAGC
     E  Y  ●

 561 TGAACAAGAATACAGGGAAAGAAGTCTCTGAAATTCGCTTCACCGCACGAGAAAAAATGACCGGGCTTGA

 631 AAGTTATGATGTCAAAATCAAAATAATGTGATTTTGTGAACATCACCCCGTGCGAGGTGATGTTCCGCTT

 701 GTTGCTAATTTAGTGACCAATCATTGGCGCTTGTGGAATTAAGCGTCGGTACAATTCCTCCGGCACCGGG

 771 CTTTGCCATACTCCCGCATACATTGCGTAACCAATCACCGCAAACATAATCCCCAGAACCAGTAGCGTCA

 841 TTAACCAGCCAGACAACGCAAAGGCTTTTTTATTTGCCGCAGGTTTTTGCAGTGAAAAGGTCAATGTTGA

 911 GGCTACCGGACATGACTCTACGCAAGTCATACAGCCGGTACATTCCACTGTTCGTACCTGAATTAATTTA

 981 TCGATCCGGGATCC
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FIG. 4. DNA sequence of plasmid pJTJ8. The analysis of the DNA sequence of the pJTJ8 plasmid revealed an open reading frame between nucleotides 268 and 498 that was designated the *sfa* gene. The stop codon is indicated (●).

TABLE 2. Fatty acid composition of strains harboring Sfa plasmids

| Plasmid | Growth temp (°C) | Fatty acids (weight %)[a] | | | | |
|---|---|---|---|---|---|---|
| | | 16:0[b] | 16:1[c] | 18:0 | 18:1 | SFA/UFA[d] ratio |
| pBS | 37 | 47.5 | 27.4 | 2.7 | 18.3 | 1.10 |
| pSJ139 | 37 | 28.7 | 19.0 | 4.3 | 42.3 | 0.54 |
| pSJ139[e] | 37 | 29.4 | 19.2 | 4.1 | 42.9 | 0.54 |
| pBS | 30 | 40.4 | 29.0 | 2.0 | 25.3 | 0.78 |
| pSJ139 | 30 | 24.8 | 21.2 | 4.0 | 45.6 | 0.43 |
| pBS | 42 | 53.6 | 18.4 | 9.6 | 13.8 | 1.96 |
| pSJ139 | 42 | 38.2 | 22.6 | 4.0 | 31.5 | 0.78 |

[a] Weight percents were calculated from duplicate samples. Strain C600 containing the indicated plasmid was grown in rich medium and harvested at $5 \times 10^8$ cells per ml.
[b] Number of carbon atoms:number of double bonds.
[c] Unsaturated fatty acids include their cyclopropane derivatives.
[d] SFA, saturated fatty acid; UFA, unsaturated fatty acid.
[e] An independent isolate of C600/pSJ139.

wild-type FabA protein at 30°C and the FabA6 protein was completely inactive at 42°C. We performed several purifications of the FabA6 protein in an attempt to isolate the protein in a more active form at the lower temperature but were not successful. These data demonstrate that the *fabA6* encodes a catalytically compromised dehydratase and establish that the mutation in the *fabA6* allele does result in a defect in the intrinsic activity of the dehydratase rather than a potential defect in the association with other proteins in the pathway.

**Overexpression of *sfa* increases unsaturated fatty acid content.** The effect of *sfa* overexpression on the production of unsaturated fatty acids was examined. Plasmid pSJ139 was constructed by PCR amplification with the primers described in Materials and Methods of the region of plasmid pJTJ8 predicted to encode the *sfa* gene. This fragment was subcloned into pBS, resulting in plasmid pSJ139. Plasmid pSJ139 was an allele-specific suppressor of *fabA6*(Ts) and exhibited all of the same physiological properties attributed to pJTJ8 in Fig. 2 and 3 (data not shown). Furthermore, strain M6 [*fabA6*(Ts)] transformed with the pSJ139 plasmid possessed a normal content of unsaturated fatty acids when grown at 42°C (29.8%), illustrating that *sfa* expression restored unsaturated fatty acid content rather than permitting the cells to grow at 42°C with an abnormally low unsaturated fatty acid content. Plasmid pSJ139 was used to transform strain C600, and the fatty acid composition was determined at three different temperatures (Table 2). At 37°C, strains containing the *sfa* gene possessed a saturated/unsaturated fatty acid ratio in their membrane phospholipids of 0.54, compared with a ratio of 1.1 in the strain harboring the control plasmid. Temperature regulation of membrane unsaturated fatty acid composition and *cis*-vaccenate content was observed in both the control strain and in the strain containing pSJ139. However, the strain expressing the *sfa* gene product overproduced unsaturated fatty acids at all temperatures compared with the controls. These data indicate that the expression of Sfa does not interfere with the thermal regulation of membrane fatty acid composition but rather boosts the synthesis of unsaturated fatty acids by perturbing the mechanism that controls the basal saturated/unsaturated fatty acid ratio.

**Conclusions.** This work identifies a 77-amino-acid open reading frame, designated *sfa*, that when overexpressed leads to a significant increase in the production of unsaturated fatty acids in *E. coli*. The ability of *sfa* to increase unsaturated fatty acid synthesis accounts for the isolation of this gene as an allele-specific repressor of a temperature-sensitive mutant defective in the production of unsaturated fatty acids. There are two genes known to be required for the synthesis of unsaturated fatty acids. FabA (β-hydroxydecanoyl-ACP dehydratase) catalyzes the first step in unsaturated fatty acid synthesis by introducing the *cis* double bond. FabB (β-ketoacyl-ACP synthase I) is also essential for unsaturated fatty acid synthesis, probably because of its unique ability to elongate a key intermediate in the pathway (most likely *cis*-3-decenoyl-ACP). Since *sfa* overexpression suppresses one class of *fabA*(Ts) mutants, one idea is that the Sfa protein stimulates the activity of FabA. However, this need not be the case, since increasing FabA activity by overexpressing the *fabA* gene either with a multicopy plasmid or in strains harboring the *fabAup* promoter mutation leads to increased amounts of saturated, rather than unsaturated, fatty acids in the membrane phospholipids (6). In contrast, the increase in saturated fatty acid synthesis in *fabAup* mutants is suppressed by the overexpression of the *fabB* gene, which increases the amount of unsaturated fatty acids in the membrane phospholipids. Thus, while both *fabA* and *fabB* are required for unsaturated fatty acid synthesis, it is the level of FabB activity relative to FabA activity that plays a determinant role in establishing the basal saturated/unsaturated fatty acid ratio. Interpretation of our results on the basis of this latter idea opens the possibility that Sfa exerts its effect by increasing the activity of FabB. Additional biochemical and genetic studies are required to determine whether Sfa alters fatty acid composition by directly interacting with one of the enzymes in fatty acid synthesis or whether its effects are indirect.

## ACKNOWLEDGMENTS

We thank Pam Jackson, Robert Becklin, and Jonathan Powell for their expert technical assistance.

This work was supported by National Institutes of Health grant GM34496, Cancer Center (CORE) support grant CA 21765, and the American and Lebanese Syrian Associated Charities.

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