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A *C*-Methyltransferase Involved in Both Ubiquinone and Menaquinone Biosynthesis: Isolation and Identification of the *Escherichia coli ubiE* Gene

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Strains of *Escherichia coli* with mutations in the *ubiE* gene are not able to catalyze the carbon methylation reaction in the biosynthesis of ubiquinone (coenzyme Q) and menaquinone (vitamin K_2), essential isoprenoid quinone components of the respiratory electron transport chain. This gene has been mapped to 86 min on the chromosome, a region where the nucleic acid sequence has recently been determined. To identify the *ubiE* gene, we evaluated the amino acid sequences encoded by open reading frames located in this region for the presence of sequence motifs common to a wide variety of *S*-adenosyl-L-methionine-dependent methyltransferases. One open reading frame in this region (*o251*) was found to encode these motifs, and several lines of evidence that confirm the identity of the *o251* product as UbiE are presented. The transformation of a strain harboring the *ubiE401* mutation with *o251* on an expression plasmid restored both the growth of this strain on succinate and its ability to synthesize both ubiquinone and menaquinone. Disruption of *o251* in a wild-type parental strain produced a mutant with defects in growth on succinate and in both ubiquinone and menaquinone synthesis. DNA sequence analysis of the *ubiE401* allele identified a missense mutation resulting in the amino acid substitution of Asp for Gly_{142}. *E. coli* strains containing either the disruption or the point mutation in *ubiE* accumulated 2-octaprenyl-6-methoxy-1,4-benzoquinone and demethylmenaquinone as predominant intermediates. A search of the gene databases identified *ubiE* homologs in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Leishmania donovani*, *Lactococcus lactis*, and *Bacillus subtilis*. In *B. subtilis* the *ubiE* homolog is likely to be required for menaquinone biosynthesis and is located within the *gerC* gene cluster, known to be involved in spore germination and normal vegetative growth. The data presented identify the *E. coli* UbiE polypeptide and provide evidence that it is required for the C methylation reactions in both ubiquinone and menaquinone biosynthesis.

The isoprenoid quinone ubiquinone (coenzyme Q) is an essential component in the respiratory electron transport chain of both eukaryotes and most prokaryotes, with the exception of the gram-positive bacteria and the blue-green algae (cyanobacteria) (26, 27). In *Escherichia coli*, Q serves as a redox mediator in aerobic respiration and performs this function via reversible redox cycling between QH_2 (the hydroquinone form) and Q (12). Our understanding of the biosynthesis and function of Q in *E. coli* derives from the characterization of the *ubi* mutants, which are completely deficient in Q and unable to grow on media containing succinate as the sole carbon source (13). The Q intermediates accumulating in strains with mutations in one of the eight *ubi* genes (*ubiA* through *ubiH*) have been identified, and the chromosomal locations of the *ubi* genes have been mapped (13, 20, 46). Clones corresponding to *ubiA* (36, 43), *ubiC* (25, 34), *ubiG* (42), and *ubiH* (24) have been identified. Additionally, a probable identification of the *ubiB* gene product has been made (7, 35).

Q synthesis requires two O methylation steps and one C methylation. Recent evidence suggests that both O methylations in *E. coli* are catalyzed by the product of the *ubiG* gene (15). Lipid extracts of the Q-deficient strain AN70 (containing the *ubiE401* mutation) contain 2-polyprenyl-6-methoxy-1,4-benzoquinone (DDMQ) as the predominant Q intermediate (45). Thus, the *ubiE* gene product is required for a *C*-methyltransferase step of Q biosynthesis: the conversion of 2-polyprenyl-6-methoxy-1,4-benzoquinol ($DDMQH_2$) to 2-polyprenyl-3-methyl-6-methoxy-1,4-benzoquinol ($DMQH_2$) (45) (Fig. 1). On this basis it was postulated that the *ubiE* gene is likely to be the structural gene for *C*-methyltransferase in Q biosynthesis.

The *E. coli ubiE* gene is also required in the synthesis of menaquinone (vitamin K_2 [MK]), an isoprenoid naphthoquinone that serves as a redox mediator in some prokaryotic respiratory electron transfer chains (12). MK biosynthesis and Q biosynthesis diverge after formation of chorismate, and the two biosynthetic pathways are independent (20) with the exception of the C methylation step (41). Thus, the *E. coli* mutant strain AN70 (containing the *ubiE401* mutation) lacks both Q and MK and instead produces only demethylmenaquinone (DMK) (41). Study of the respiratory capacity of the mutant strain AN70 showed that DMK can function in anaerobic respiration with fumarate, trimethylamine *N*-oxide, or dimethyl sulfoxide as an electron acceptor but is ineffective with nitrate (41). Hence, either MK or DMK is required in anaerobic respiration with fumarate, trimethylamine *N*-oxide, or dimethyl sulfoxide, while either MK or Q is required in anaerobic respiration with nitrate. Thus, respiration under the latter conditions requires a functional *ubiE* gene product and the consequent methylation of either demethylmenaquinol ($DMKH_2$) to menaquinol (MKH_2) or $DDMQH_2$ to $DMQH_2$ (Fig. 1).

Recent analyses of methyltransferase amino acid sequences

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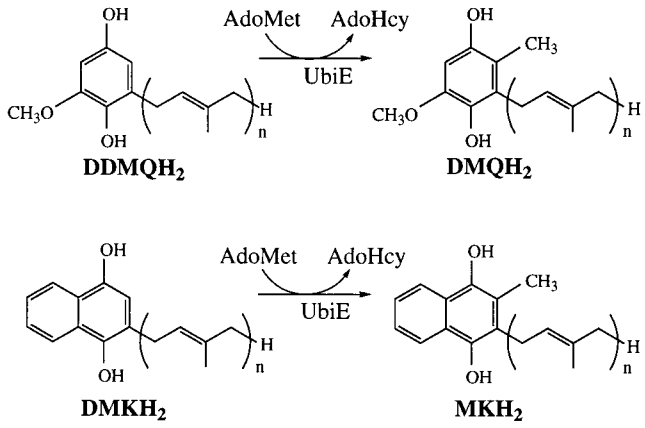

FIG. 1. *E. coli* UbiE is proposed to catalyze a carbon-methyl transfer in both Q and MK biosynthesis. The *C*-methyltransferase reaction is shown for intermediates involved in biosynthesis of Q, i.e., $DDMQH_2$ and $DMQH_2$, and for the synthesis of MKH_2 from $DMKH_2$. The methyl donor for the reaction is AdoMet, and the end product is *S*-adenosyl-L-homocysteine (AdoHcy). Intermediates are represented as quinols because the methylation is considered an electrophilic substitution reaction (27). In *E. coli* the predominant quinones are Q_8 and MK_8, and DMK_8 may also be present. n, number of isoprene units.

have identified at least three motifs common to a wide variety of enzymes that use *S*-adenosyl-L-methionine (AdoMet) as a methyl donor (17). The amino acid residues in these motifs have been found either to make contact with AdoMet or to provide scaffolding for other residues that contact this cofactor, as evidenced by comparison of the crystal structures of both DNA and catechol *O*-methyltransferases (33). It is possible to use the methyltransferase motifs to predict methyltransferases encoded by hypothetical open reading frames (ORFs) in genomic and cDNA databases (17). Thus, we sought to use these motifs to identify the putative UbiE methyltransferase based on the map location of the *ubiE* gene (45) and the reported DNA sequence of the *E. coli* chromosome in this region (7). Our search led to the identification of an uncharacterized ORF (*o251*) that encoded all three methyltransferase motifs and mapped close to the reported location of *ubiE*. The present study identifies *o251* as the *ubiE* gene and shows that it is essential for the synthesis of both Q and MK in *E. coli.*

MATERIALS AND METHODS

Strains and growth media. The *E. coli* strains used in this study are listed in Table 1. Bacteria were grown in Luria-Bertani (LB) broth with vigorous shaking at 37°C unless otherwise specified. The AN70 *ubiE* mutant was grown in brain heart infusion media (Difco) to reduce its reversion frequency. Defined medium containing either succinate (SDM) or glucose contained 0.5% Casamino Acids supplement and was prepared as described previously (28). The growth of strains JC7623 and JC7623Δ4-1 requires the addition of 60 mg of proline per liter of SDM. When required, ampicillin and kanamycin were added to a final concentration of 50 μg/ml. All solid media contained 2% Difco Bacto agar.

Plasmid construction. The following primers were used to PCR amplify the *o251* ORF from JC7623 genomic DNA and contained *Mlu*I linkers (underlined): pUBE1 (5′CCCACGCGTTGTATGGTGGATAAGTCACAAGA3′) and pUBE2 (5′CCCACGCGTTCAGAACTTATAACCACGATGCA3′). Oligonucleotide primers were synthesized by the phosphoramidite method on a Gene Assembler II instrument (Pharmacia Biotech, Inc.). The 756-bp resulting product was digested with *Mlu*I and ligated into the *Mlu*I site of pQM to form pUE3 and pUE6. The pUE3 construct contains the *o251* ORF downstream of the *Saccharomyces cerevisiae CYC1* promoter and in frame with the mitochondrial amino terminus-targeting sequence present in pQM (15). The targeting sequence was used in a separate study to investigate the ability of *o251* to restore growth on glycerol in yeast mutants deficient in the same biosynthetic step (3). The *o251* ORF was expressed in both orientations with pUE3 in the sense direction and pUE6 in the antisense direction. Previous work has shown that the *S. cerevisiae CYC1* promoter functions in *E. coli* (15).

Construction of *ubiE* null mutants. The following primers were used to PCR amplify a segment of JC7623 genomic DNA containing the *o251* (*ubiE*) ORF with 1.5 kb of flanking sequence on either side of the coding sequence: pUE1-3 (5′CGGGATCCACGCAGTTATTGCGTTGGTGGGTGTGGC3′, from 70428 to 70455, corresponding to the site 1,472 bp upstream of the *o251* ORF initiator codon) and pUE2-3 (5′CGGGATCCGCTTACGAAGATGTTGCCAGGGTG C3′, from 74183 to 74159, corresponding to the site 1,475 bp downstream of the *o251* stop codon) (7). Both primers contained *Bam*HI linkers (underlined). The resulting PCR product (3.76 kb) was digested with *Bam*HI, end repaired, and blunt-end ligated into the *Not*I site of pBlueScript II SK(+) (Stratagene) to generate pBo251-1. The *ubiE* locus in pBo251-1 was disrupted by complete digestion with *Not*I (at a site 489 bp downstream of the *ubiE* ATG codon) to generate a linear plasmid. A 1.2-kb *Bam*HI fragment of the Tn*5* kanamycin resistance cassette of pUC4-KIXX (Pharmacia Biotech) was blunt-end ligated into the linear plasmid to generate the construct pBo251-Kan. The construct was linearized with *Kpn*I and transformed into *E. coli* JC7623. Kan^r and Amp^s transformants were selected for their inability to grow on SDM (with proline), and they were designated JC7623Δ4. The disruption and the orientation of the kanamycin cassette in JC7623Δ4-1 were verified by both PCR and Southern hybridization.

Lipid extraction and analysis of *E. coli* Q_8, Q_8 intermediates, DMK_8, and MK_8. An overnight culture of *E. coli* AN70 was inoculated into 1 liter of brain heart infusion broth containing 0.65 μCi of *p*-[U-^{14}C]hydroxybenzoic acid (365 Ci/mol, prepared as described previously [29]). The culture was incubated at 37°C with shaking (350 rpm) for 16 to 20 h and then subjected to lipid extraction as described previously (29). The lipid extracts were resuspended in 1 ml of hexane/liter of culture. Radiolabeled lipid extracts were first separated by C_{18} SepPak chromatography (Waters) with the elution solvents acetonitrile, acetonitrile-isopropanol (85:15), acetonitrile-isopropanol (70:30), and isopropanol as described previously (15). Fraction 2 contained 60% of the total radioactivity and was further purified by reverse-phase high-performance liquid chromatography (HPLC) with an acetonitrile-isopropanol mobile phase. Following sample injection into 100% acetonitrile, the percentage of isopropanol was increased linearly at 1% per min to 50% as described previously (15). The separation was monitored at 272 nm, and 1-ml fractions were collected at a flow rate of 1 ml/min. The radioactivity in each fraction was determined by scintillation counting. Fractions containing ^{14}C-radiolabeled material, as well as the unlabeled fractions 23 through 26, were dried under a stream of nitrogen, resuspended in hexane, and subjected to mass spectrometry analysis as described previously (29). The same procedures were performed on lipid extracts prepared from cultures of AN70:: pUE6 (in LB plus ampicillin broth), JC7623 (in LB broth), and JC7623Δ4-1 (in LB broth), except only the radiolabeled fractions for both JC7623 and JC7623Δ4-1 were subjected to mass spectral analysis. A standard of Q_8 (Q with eight isoprene units) was not available, so the Q_8 that formed in the wild type and in the rescued mutant strains was identified based on its elution profile relative to that of Q_7 and Q_9 and by mass spectral analysis of the isolated compound. Similarly, both DMK_8 and MK_8 were isolated from the rescued strain and identified by mass spectroscopy.

Sequence analysis. DNA sequence analysis of the *ubiE* mutant allele in AN70 was determined by dideoxynucleotide chain termination (32) with a Sequenase kit (U.S. Biochemicals) and α-^{35}S-dATP (1,069 Ci/mmol; NEN Research Products). The DNA was sequenced from a PCR-amplified product of AN70 genomic DNA with the following primers for amplification: pAN70-1 (5′TTCATCGAT GACATGTCCGC3′, from 71559 to 71578, corresponding to the site 350 bp upstream of the *ubiE* ATG codon) and pAN70-2 (5′AATACTTTACCCAGCA GACG3′, from 72806 to 72787, corresponding to the site 104 bp downstream of the *ubiE* stop codon) (7). Primer pAN70-3 (5′AAAAACTCTCTTCAGGGCG C3′, from 71628 to 71647, corresponding to the site 280 bp upstream of the *ubiE* ATG codon) and primer pAN70-4 (5′CGTTTTCAGCGCGGGTGAGC3′, from 72777 to 72758, corresponding to the site 74 bp downstream of the *ubiE* stop codon) (7) were used in the initial sequencing reactions; subsequent primers were made based on the sequences obtained from the previous primers, and the amplified segment was sequenced in both directions.

TABLE 1. Genotypes and sources of *E. coli* strains

Strain	Genotype	Reference
AN70	*ubiE401*	45
JC7623	F$^-$ *thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-15 tsx-33 rpsL31 supE44 recB21 recC22 sbcB15 sbcC201*	18
JC7623Δ4-1	JC7623, *ubiE*::Kan^r	This work

RESULTS

Identification of the *o251* ORF as *ubiE*. The *E. coli* DNA sequence reported by Daniels et al. (7) spans the region of the *E. coli* genetic map determined to contain *ubiE* (45). A search of the reported ORFs in this segment of the *E. coli* chromosome identified *o251*, which encoded the three methyltransferase motifs identified by Kagan and Clarke (17). Such motifs are present in a large family of methyltransferases that use AdoMet as the methyl donor and have also been identified in the eukaryotic Coq3 *O*-methyltransferase in Q biosynthesis (5, 19), in the *E. coli* UbiG *O*-methyltransferase (42), and in the sequence encoded by *ERG6*, the presumed structural gene for AdoMetΔ 24-sterol-*C*-methyltransferase in ergosterol synthesis (14, 30). None of the other reported ORFs in this region were found to encode methyltransferase motifs. Thus, the *o251* ORF is a candidate for the *ubiE* gene. To test this idea, the constructs pUE3 and pUE6, which contained the *o251* ORF in the *E. coli* expression plasmid (pQM), were prepared and were used to transform *E. coli* AN70, which carries the mutant allele *ubiE401* and fails to grow on SDM. Transformants were tested for their restored ability to grow on SDM. Both constructs pUE3 and pUE6 were able to rescue growth of *E. coli* AN70 on SDM, whereas the vector control plasmid pQM failed to rescue AN70 (data not shown). This result indicated that expression of *o251* from either the *CYC1* promoter (pUE3) or a cryptic promoter in the vector pQM (pUE6) was responsible for rescuing AN70 and suggested that *o251* corresponds to the *ubiE* ORF. We then utilized a segment of DNA containing *o251* to prepare a chromosomal disruption mutant containing the Tn*5* kanamycin resistance cassette 489 bp downstream of the *o251* initiator codon. The resulting mutant, JC7623Δ4-1, failed to grow on SDM (data not shown). These results show that the phenotype of the JC7623Δ4-1 disruption mutant is similar to that of the mutant strain AN70 with respect to growth on succinate and confirm the identification of *o251* as *ubiE*.

Sequence analysis of the *ubiE* mutant allele in strain AN70. To identify the defect in the *ubiE401* mutant allele, a DNA segment encompassing the *o251* coding region plus 350 bp of 5′-flanking and 104 bp of 3′-flanking sequence was PCR amplified from AN70 and sequenced in both directions. Sequence analysis identified a single base change of G to A at position 424, resulting in a change from Gly to Asp at amino acid position 142 (Fig. 2). The analysis also corrected the ambiguities found in the published sequence as indicated in Fig. 2. The presence of a mutation within the *o251* ORF in the AN70 mutant is strong evidence that *o251* is indeed the *ubiE* gene. Furthermore, the rescue of the AN70 (*ubiE401*) point mutant by a plasmid containing *o251* (data not shown) indicates that there are no other mutations in AN70 affecting growth on succinate.

Purification and identification of the Q_8, Q_8 intermediates, DMK_8, and MK_8 in *ubiE* mutant and wild-type *E. coli* strains. The mutant strain AN70 was shown to lack both Q_8 and MK_8 and to accumulate DDMQ and DMK_8 (41, 45). Thus, it was important to determine whether both Q_8 synthesis and MK_8 synthesis were restored in AN70 harboring the wild-type *ubiE* gene. The construct pUE6 was chosen for these studies because UbiE is expressed in an unmodified form with pUE6 and growth of the rescued AN70 transformants is more vigorous with pUE6 than with pUE3 (data not shown). Q and Q intermediates were radiolabeled by supplementing the growth media with *p*-[U-^{14}C]hydroxybenzoic acid, and lipid extracts were prepared and fractionated by reverse-phase HPLC as described in Materials and Methods. Analysis of *p*-[U-^{14}C]hydroxybenzoic acid-radiolabeled lipid extracts from AN70 showed a large peak of radioactivity in fractions 16 and 17 (data not shown), and the elution position suggested it was slightly more polar than the Q_8 standard (Fig. 3A). Electron impact mass spectral analysis of fractions 16 and 17 identified ions corresponding to $DDMQH_2$ (M + 2 = $C_{47}H_{72}O_3$: 684.548147; observed mass, 684.547192; ppm, 1.4) and DDMQ (M = $C_{47}H_{70}O_3$: 682.532497; observed mass, 682.531444; ppm, 1.5). As expected for a quinone-containing intermediate, both M + 2 and M ions were present in each fraction (data not shown). In the same lipid extract, mass spectral analysis of fractions 23 and 24 identified DMK_8 (M = $C_{50}H_{70}O_2$: 702.5376; observed mass, 702.5372; ppm, 0.6). Fractions 25 and 26 were also subjected to mass spectral analysis, but no MK_8 was detected (Fig. 3A). Transformation of AN70 with pUE6 restored the synthesis of both Q_8 and MK_8 as indicated by the UV absorbance of material in fractions 19 and 26, respectively (Fig. 3B). Mass spectral analysis confirmed the presence of Q_8 and revealed M and M + 2 ions at 726 and 728 as well as the expected tropylium and pyrylium ions at 197 and 235 (8). The presence of DMK_8 in fractions 23 and 24 (M = $C_{50}H_{70}O_2$:

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ATGAAGAGTATCGACTCTGGTCGGTTCCGGAGCAGCCGAATGATGAAGCTT  -132
ATCAACGCGATGATGAATATAATCAGCAGTCGCGCTAGCCCATTGGGAGTA   -81
GTTAAGCCGGGTAGAAATCTAGGGCATCGACGCCCAATCTGTTACACTTCT   -31
GGAACAATTTTTTGATGAGCAGGCATTGAGATGGTGGATAAGTCACAAGAA    21
                              M  V  D  K  S  Q  E       7
ACGACGCACTTTGGTTTTCAGACCGTCGCGAAGGAACAAAAAGCGGATATG   72
T  T  H  F  G  F  Q  T  V  A  K  E  Q  K  A  D  M     24
GTCGCCCACGTTTTCCATTCCGTGGCATCAAAATACGATGTCATGAATGAT   123
V  A  H  V  F  H  S  V  A  S  K  Y  D  V  M  N  D     41
TTGATGTCATTTGGTATTCATCGTTTGTGGAAGCGATTCACGATTGATTGC   174
L  M  S  F  G  I  H  R  L  W  K  R  F  T  I  D  C     58
AGCGGCGTACGCCGTGGGCAGACCGTGCTGGATCTGGCTGGTGGCACCGGC   225
S  G  V  R  R  G  Q  T  V  L  D  L  A  G  G  T  G     75
GACCTGACAGCGAAATTCTCCCGCCTGGTCGGAGAAACTGGCAAAGTGGTC   276
D  L  T  A  K  F  S  R  L  V  G  E  T  G  K  V  V     92
CTTGCTGATATCAATGAATCCATGCTCAAAATGGGCCGCGAGAAGCTGCGT   327
L  A  D  I  N  E  S  M  L  K  M  G  R  E  K  L  R    109

                                          SS
AATATCGGTGTGATTGGCAACGTTGAGTATGTTCAGGCGAACGCTGAGGCG   378
N  I  G  V  I  G  N  V  E  Y  V  Q  A  N  A  E  A    126

                                             A
CTGCCGTTCCCGGATAACACCTTTGATTGCATCACCATTTCGTTTGGTCTG   429
L  P  F  P  D  N  T  F  D  C  I  T  I  S  F  G  L    143
CGTAACGCCACCGACAAAGATAAAGCACTGCGTTCAATGTATCGCGTGCTG   480
R  N  V  T  D  K  D  K  A  L  R  S  M  Y  R  V  L

      A
AAACCCGGCGGCCGCCTGCTGGTGCTTGAGTTCTCGAAGCCAATTATCGAG   531
K  P  G  G  R  L  L  V  L  E  F  S  K  P  I  I  E    177

                                          SS
CCGCTGAGCAAAGCCTATGATGCATACTCCTTCCATGTGCTGCCGCGTATT   582
P  L  S  K  A  Y  D  A  Y  S  F  H  V  L  P  R  I    194
GGCTCACTGGTCGCGAACGACGCCGACAGCTACCGTTATCTGGCAGAATCC   633
G  S  L  V  A  N  D  A  D  S  Y  R  Y  L  A  E  S    211
ATCCGTATGCATCCCGATCAGGATACCCTGAAAGCCATGATGCAGGATGCC   684
I  R  M  H  P  D  Q  D  T  L  K  A  M  M  Q  D  A    228
GGATTCGAAAGTGTCGACTACTACAATCTGACGGCAGGGGTTGTGGCGCTG   735
G  F  E  S  V  D  Y  Y  N  L  T  A  G  V  V  A  L    245
CATCGTGGTTATAAGTTCTGA                                 756
H  R  G  Y  K  F  .                                   252
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FIG. 2. Corrected nucleotide sequence and deduced protein sequence of the *ubiE* gene. The amino acid residues are indicated under the first letter of each codon. The predicted initiator methionine codon of the *ubiE* gene is at position +1. Ambiguous nucleotides (outlined S indicates C or G) present in the published sequence (GenBank accession number M87049; ORF *o251*) were resolved, and the corrections are indicated at positions 369, 370, 570, and 571. The outlined A at nucleotide 424 designates the single base pair change (A instead of G) present in the mutant *ubiE401* gene and predicts Asp_{142} instead of Gly. The "Δ" at nucleotide 489 corresponds to the insertion of the kanamycin cassette at the *Not*I site, creating the *ubiE*::Kan^r disruption mutant JC7623Δ4-1. Transcription from the kanamycin cassette proceeds in the direction opposite to that of *ubiE*. The three underlined sequences correspond to the conserved methyltransferase motifs I, II, and III (17).

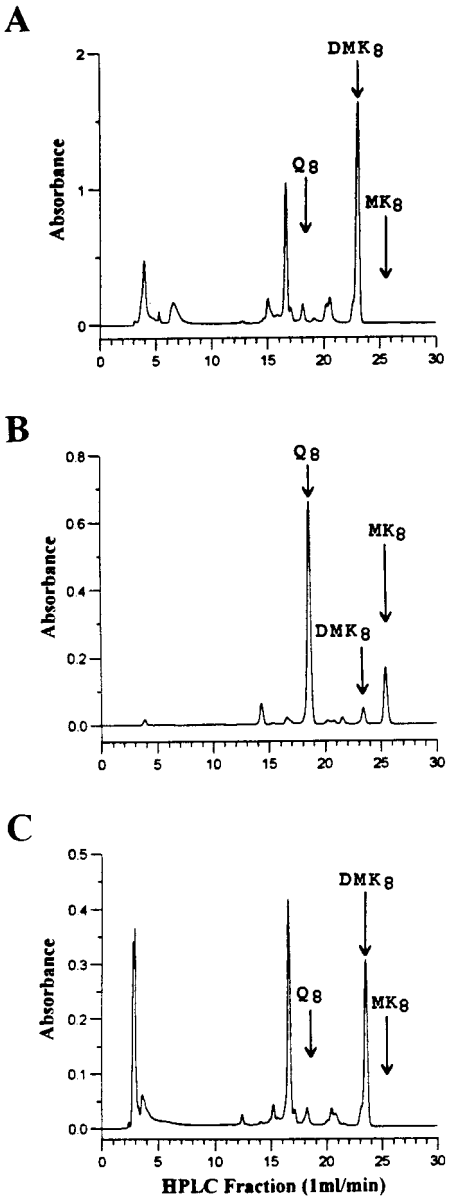

FIG. 3. Expression of *o251* in *E. coli* is required for synthesis of both Q_8 and MK_8. Lipid extracts from *E. coli* strains labeled with *p*-[U-^{14}C]hydroxybenzoic acid were separated by reverse-phase HPLC. Absorbance (272 nm) is depicted for AN70 harboring the *ubiE401* mutation (A), AN70 rescued with pUE6 (B), and the *ubiE* null mutant JC7623Δ4-1 (C).

702.5376; observed mass, 702.5392; ppm, 2.2) and MK_8 in fractions 25 and 26 (M = $C_{51}H_{72}O_2$: 716.5532; observed mass, 716.5537; ppm, 0.6) was confirmed by mass spectroscopy.

These results are consistent with the identification of *o251* as *ubiE* and predict that both DDMQ and DMK should accumulate in the putative *ubiE* disruption mutant JC7623Δ4-1. Analysis of extracts from JC7623Δ4-1 (Fig. 3C) showed the presence of DDMQ in fraction 17 (M = $C_{47}H_{70}O_3$: 682.532497; observed mass, 682.531690; ppm, 1.2; M + 2 = $C_{47}H_{72}O_3$: 684.548147; observed mass, 684.544417; ppm, 5.4). Mass spectral analysis of fractions 18, 19, and 21 revealed no detectable levels of Q_8. The UV absorbance profile in Fig. 3C mirrors that shown in Fig. 3A and shows that mutants containing either the *ubiE* point mutant allele or the *ubiE* disruption allele accumulate DMK (fraction 23) and lack MK (fraction 26). Corresponding analysis of the parental strain, JC7623, generated a profile similar to that shown in Fig. 3B and produced the expected ions corresponding to Q_8 as well as UV-absorbing materials in fractions 23 and 26 that coeluted with DMK and MK standards, respectively (data not shown).

DISCUSSION

The results presented here provide strong evidence for the identification of the *ubiE* gene in *E. coli. E. coli ubiE* mutants were shown to be defective in a *C*-methyltransferase step of both Q and MK biosynthesis and to accumulate the intermediates DDMQ and DMK (41, 45). It was hypothesized that *ubiE* comprised the structural gene for *C*-methyltransferase. The present work investigated whether an ORF (*o251*) contained within the sequence reported by Daniels et al. (7) as part of the *E. coli* genome project corresponds to *ubiE*. We identified the *o251* ORF as a candidate gene based on its location in a region of the *E. coli* chromosome corresponding to the genetic map position of 86 min for *ubiE* (7, 45) and because it was the only ORF in this region that encoded the three methyltransferase motifs identified by Kagan and Clarke (17). Several lines of evidence presented here confirm the identity of *o251* as *ubiE*. The expression of *o251* in AN70 (a strain containing the *ubiE401* mutation) restored both the growth of this strain on succinate and its ability to synthesize both Q_8 and MK_8. This rescue by *o251* provides evidence that the defects in both Q and MK synthesis previously observed in AN70 result from a single mutation (20). Disruption of *o251* in a *ubiE*$^+$ parental strain produced a mutant with a phenotype similar to that of AN70, including defects in growth on succinate and in both Q_8 and MK_8 synthesis. The disruption mutant also accumulated DDMQ as the predominant Q intermediate and DMK as the predominant MK intermediate. These results show that *ubiE* is required for the C methylation step in the synthesis of both Q and MK.

Further evidence that *ubiE* is *o251* is the presence of a missense mutation in the *ubiE401* allele resulting in the amino acid substitution of Asp for Gly_{142}. A database search identified several UbiE homologs in both prokaryotic and eukaryotic species and indicates that this substitution occurs in a region with a high degree of sequence identity (Fig. 4). The position of this substitution immediately follows methyltransferase motif II (Fig. 2). The crystal structure determination of catechol *O*-methyltransferase (COMT) identifies the post-motif II region as part of the active site and in contact with both the catechol substrate and AdoMet (40). The sequence and structure comparisons of three AdoMet-dependent methyltransferases of known structure (COMT and the DNA methyltransferases M.*Hha*I and M.*Taq*I) indicate a conserved catalytic domain for this general class of enzymes (33). It is thus reasonable to postulate that this conserved catalytic domain is also present in the UbiE polypeptide. The alignment of the predicted UbiE amino acid sequence with the well-characterized COMT sequence over methyltransferase motifs I, II, and III is shown in Fig. 5. It is important to note that Gly_{142} of UbiE occurs in the same relative position as W_{143} of COMT and that the latter residue contacts both the catechol substrate and the adenine ring of AdoMet. Thus, it is tempting to speculate that the Gly_{142}-to-Asp mutation in *ubiE401* occurs in a critical region of the UbiE polypeptide involved in binding AdoMet and/or DDMQ.

The phenotype of the AN70 point mutant was similar to that of the disruption mutant (JC7623Δ4-1). However, unlike the point mutant, the disruption mutant was not rescued by the

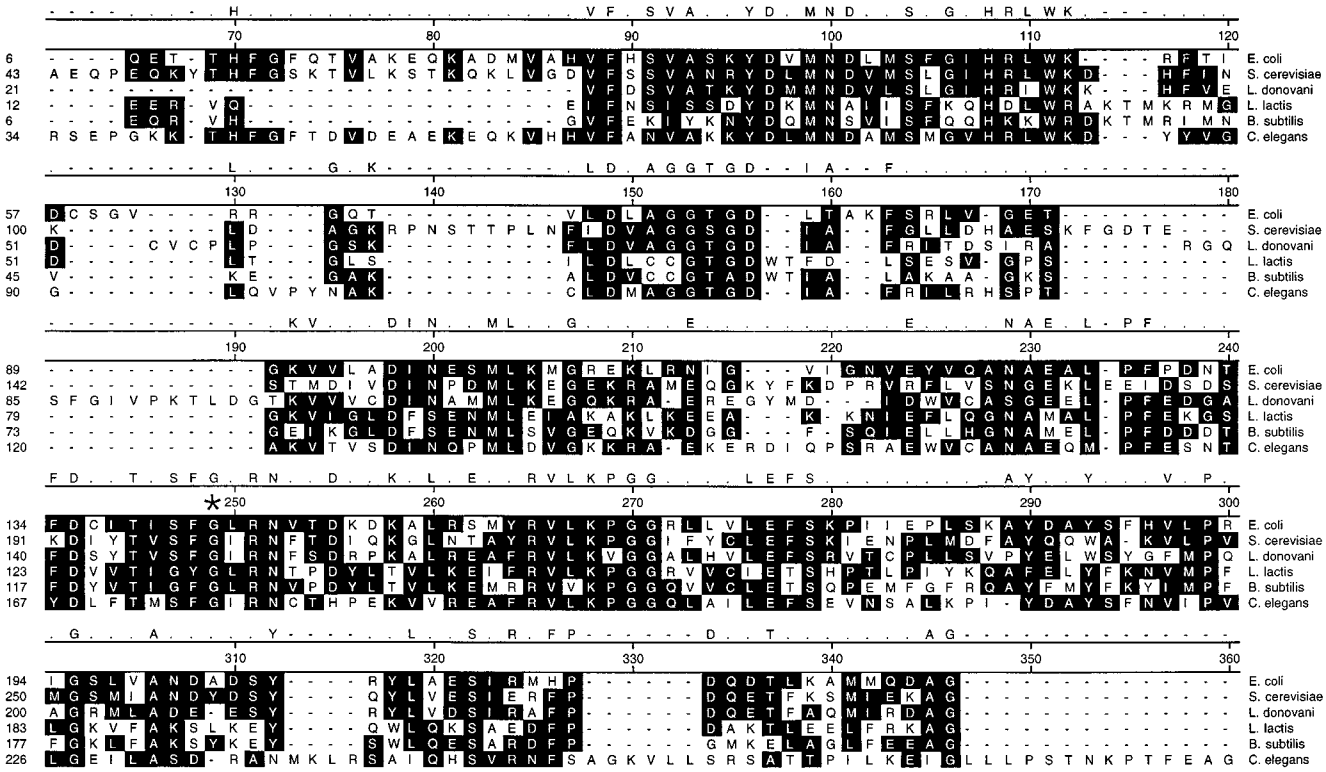

FIG. 4. UbiE homologs in prokaryotic and eukaryotic cells. The predicted *E. coli* UbiE protein sequence is shown in alignment with probable homologs identified in *S. cerevisiae* (40% identity over 239 amino acids; GenBank accession number P49017), *L. donovani* (43% identity over 213 amino acids; B48583), *B. subtilis* (36% identity over 200 amino acids; P31113), *L. lactis* (37% identity over 200 amino acids; P49016), and *C. elegans* (42% identity over 207 amino acids; P34666). The alignments were generated from DNAstar MegAlign by using the PAM 250 table and the Jotun Hein method. The shaded residues match the consensus within two distance units. The residues above the ruler represent the amino acid identities among a majority of the sequences. The asterisk indicates the position of the Gly_{142}-to-Asp change predicted from the sequence of the mutant *ubiE* allele of AN70.

expression of *ubiE* on a plasmid (neither the pUE6 or pUE3 plasmid restored growth on succinate [data not shown]). This lack of rescue might result from the effect of the disruption on the expression of one or both of the ORFs downstream of *ubiE*/*o251*. Sequence analysis by Daniels et al. (7) suggested that *o251* is the promoter-proximal ORF of an operon and is followed by ORFs *o200* and *o121*. Thus, disruption of *ubiE*/*o251* by the kanamycin cassette (the transcription of the kanamycin cassette is oriented opposite to that of *ubiE*) might be expected to impair expression of the downstream ORFs *o200* and *o121* as well. Expression of these ORFs may be required for growth on succinate. However, a search of the databases with these ORFs does not reveal sequence homology with genes encoding any known class of proteins.

A BLAST search of GenBank with the *E. coli* UbiE amino acid sequence identified homologs in *Bacillus subtilis* (44) and *Lactococcus lactis* (11) with 36 and 37% sequence identity to *E. coli* UbiE over 200 amino acids, respectively (Fig. 4). Gram-positive bacteria, such as *B. subtilis*, do not synthesize Q but instead use MK, which is an essential component of the electron transport chain (37). Thus, it seems likely that the UbiE homologs in *L. lactis* and *B. subtilis* are involved in the methylation of $DMKH_2$ to MKH_2 (Fig. 1). In *B. subtilis*, the UbiE homolog is encoded by the second of three ORFs contained in the *gerC* gene cluster (accession number P31113). Yazdi and Moir (44) performed a careful characterization of the *gerC58* mutants of *B. subtilis* and uncovered two phenotypes: in suppressed strains harboring an unlinked mutation, spores showed

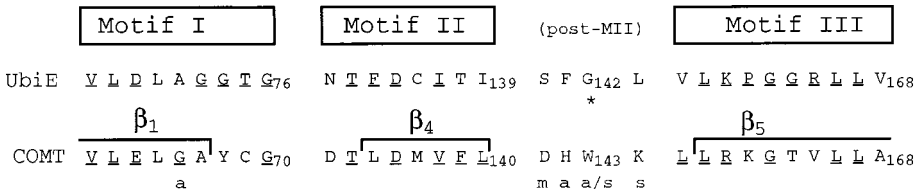

FIG. 5. Alignment of the *E. coli* UbiE and rat COMT amino acid sequences across methyltransferase motifs I, II, and III. The three motifs of sequence similarity present in AdoMet-dependent methyltransferases are shown; underlined residues designate a match with the consensus amino acids identified by Kagan and Clarke (17). The carboxy-terminal residue of each motif is numbered and indicates the position of motifs I, II, and III relative to the linear amino acid sequence of the polypeptide chain. The post-motif II region (post-MII) consists of the amino acids immediately following motif II. The secondary-structure elements in the crystal structure of the rat soluble COMT (β_1, β_4, and β_5) (40) and the important active-site residues involved in binding of ligands are indicated. a, AdoMet; m, magnesium; s, substrate. The asterisk indicates the position of the Gly_{142}-to-Asp change predicted from the sequence of the mutant *ubiE* allele of AN70.

a temperature-sensitive germination defect in response to alanine-induced germination, and in unsuppressed strains, colonies exhibited very slow growth (the tiny phenotype) and were sporulation defective. It is possible that the phenotype(s) of the *gerC58* mutant is due to a defect in synthesis of MK. In *B. subtilis*, MK has been shown to be necessary for sporulation, and its formation is highly regulated (9, 10). Furthermore, insufficient MK biosynthesis results in impaired growth (a small-colony phenotype) and defects in sporulation (9, 10, 21, 22). Sporulation is particularly sensitive to decreases in MK; when the MK concentration falls to 1/10 of the normal concentration (where respiration and cytochromes are at normal levels), sporulation is completely abolished (9). Thus, it seems likely that the UbiE homolog (encoded by *gerC2*) in *B. subtilis* is required for production of MK. The levels of DMK in gram-positive organisms are generally quite low (6), and its possible function has apparently not been investigated. Although the *gerC58* mutation has been mapped to *gerC3*, the third ORF in the *gerC* gene cluster (unpublished data cited in reference 23), it is interesting that this ORF corresponds to a polyprenyl synthase (2) and corresponds to a family of enzymes including farnesyl-, geranylgeranyl-, hexaprenyl-, and octaprenyl-diphosphate synthases (1, 4). Thus, *gerC3* itself is likely to be essential for the production of the polyprenyl moiety on MK. It will be interesting and important to determine whether the phenotype associated with the *gerC58* mutant is due to defects in MK synthesis.

UbiE homologs were also identified in several eukaryotic species, including the products of hypothetical ORFs in *Caenorhabditis elegans* and *S. cerevisiae* and of ORF A41 in *Leishmania donovani* (16), which produces Q_9 (31). The mRNA level of the UbiE homolog in *L. donovani* was found to be about twofold higher in the intracellular, nonmotile amastigote form of the parasite than the RNA levels isolated from the extracellular, flagellated promastigote form (16). In studies to be reported (3), the gene encoding the *S. cerevisiae* UbiE homolog has been identified as *COQ5* and has been found to be allelic with the original mutant *coq5* (38, 39). Thus, it is likely that the UbiE homologs in eukaryotes function similarly to yeast Coq5 and are involved in production of Q. The data reported here show that *o251* is the *ubiE* gene in *E. coli* and is likely to encode *C*-methyltransferase for the production of both Q and MK. Further characterization of UbiE activity will require chemical amounts of substrate and purified protein.

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