The *ispB* Gene Encoding Octaprenyl Diphosphate Synthase Is Essential for Growth of *Escherichia coli*

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Received 12 February 1997/Accepted 3 March 1997

The *Escherichia coli* *ispB* gene encoding octaprenyl diphosphate synthase is responsible for the synthesis of the side chain of isoprenoid quinones. We tried to construct an *E. coli* *ispB*-disrupted mutant but could not isolate the chromosomal *ispB* disrupted mutant unless the *ispB* gene or its homolog was supplied on a plasmid. The chromosomal *ispB* disruptants that harbored plasmids carrying the *ispB* homologs from *Haemophilus influenzae* and *Synechocystis* sp. strain PCC6803 produced mainly ubiquinone 7 and ubiquinone 9, respectively. Our results indicate that the function of the *ispB* gene is essential for normal growth and that this function can be substituted for by homologs of the *ispB* gene from other organisms that produce distinct forms of ubiquinone.

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*Escherichia coli*, a member of the gram-negative and facultative anaerobic group of bacteria, usually obtains energy for growth through respiration under aerobic and anaerobic conditions, in addition to energy obtained from glycolysis (8, 10). In the respiratory chain of *E. coli*, two types of quinones, ubiquinone 8 (UQ-8) and menaquinone 8 (MK-8), are essential components (3, 5, 8, 10). UQ-8 is necessary for the transfer of electrons from NADH to succinate in the electron transfer system that has molecular oxygen as the final electron acceptor. MK-8 functions for the transfer of electrons from formate in the anaerobic electron transfer system which uses nitrate as the final electron acceptor. While *E. coli* has both UQ-8 and MK-8, some microorganisms contain only one type of quinone; e.g., *Bacillus* species produce only MK and *Acetobacter* species generally produces only UQ (3, 5). Why does *E. coli* synthesize two kinds of quinones when other bacteria can subsist on only one? To address this question, mutants deficient in the synthesis of UQ, MK, or both have been isolated (19). *ubi* and *men* mutants are respiration defective under aerobic and anaerobic conditions, respectively (10, 19, 20). A strain defective in both *ubi* and *men* has been constructed and found to grow very slowly (19). However, it appears likely that the *ubi* mutation was leaky in this strain, as a small amount of UQ could still be detected (19). We have taken a different approach by isolating a mutant with a deletion of the *ispB* gene, which encodes octaprenyl diphosphate synthase (1). This enzyme is responsible for the synthesis of the side chain of both UQ and MK, and strains defective in this enzyme should not be able to synthesize active forms of UQ or MK (1). However, we found that it was impossible to obtain an *ispB* deletion mutant unless the *ispB* gene or its homolog was supplied on a plasmid. Thus, we suggest that the *ispB* gene is essential for the normal growth of *E. coli*.

**Construction of an *ispB*-disrupted mutant.** To investigate the function of the *ispB* gene, a plasmid (pTC2) used to disrupt this gene was constructed by inserting the chloramphenicol acetyltransferase (*cat*) gene into the *ispB* gene (Fig. 1). We attempted to obtain chloramphenicol-resistant strains by transforming strain FS1576 (recD) (15) with the linearized *KpnI-HindIII* fragment from pTC2, but no Cm< sup> R</ sup> transformants were obtained. However, when FS1576 harboring a plasmid (pKA3) containing the *ispB* gene was used as a host cell, we obtained many Cm< sup> R</ sup> transformants. Several transformants were examined for proper replacement of the chromosomal *ispB* gene. One strain, designated KO229 (*ispB::Cm< sup> R</ sup>*), was confirmed to have the correct gene disruption by Southern blot analysis (data not shown). We attempted to cure strain KO229 of pKA3 to find out whether growth of KO229 is or is not dependent on pKA3. Strain KO299 harboring pKA3 (spectinomycin resistant) was subcultured five times on nonselective Luria (L) medium and then plated on L agar medium. When 1,000 colonies were replica plated on L agar medium containing 50 µg/ml, all of the colonies were spectinomycin resistant and a strain of KO229 that had lost pKA3 was never isolated. KO229 maintained pKA3, which contains the *ispB* gene, even under the nonselective conditions. KO229 harboring pKA3 showed growth characteristics similar to those of the wild-type strain, and the production of UQ-8 was normal (data not shown). To test further the importance of the *ispB* gene for the growth of *E. coli*, we recloned the *ispB* gene into plasmid pSI029, which has a temperature-sensitive (*ts*) replication origin (18), to yield plasmid pSI7 (Fig. 1). Strain KO229 harboring only pSI7 was obtained by swapping pKA3 for pSI7. KO229 harboring pSI7 could grow at 30°C (permissive temperature), while the same strain could not grow at 43°C (restriction temperature) (data not shown). This result indicates that the *ispB* gene is essential for the growth of *E. coli*.

**Complementation of the *E. coli* *ispB* disruptant with homologs from *Haemophilus influenzae* and *Synechocystis* sp. strain PCC6803.** To further investigate the significance of the *ispB* gene in *E. coli*, we used *ispB* homologs from *H. influenzae* (7) and *Synechocystis* sp. strain PCC6803 (9). Recently, the complete genomic sequences of *H. influenzae* and *Synechocystis* sp. strain PCC6803 were determined and *ispB* homologs with 64.9 and 34.5% identity to that of *E. coli* were reported for *H. influenzae* (7) and *Synechocystis* (9). The *ispB* gene from *Synechocystis* sp. strain PCC6803 contains two insertions at the N-terminus of the mature protein that are absent in the *E. coli* gene. We therefore constructed a deletion mutant of the *ispB* gene from *Synechocystis* sp. strain PCC6803 (pSI7Δ) that contained the insertions present in the *E. coli* gene. Strain KO229 harboring pSI7Δ showed similar growth characteristics to KO229 harboring pSI7 (Fig. 2). These results indicate that the *ispB* homolog from *Synechocystis* sp. strain PCC6803 can complement the *ispB* disruptant. The *ispB* homolog from *H. influenzae* has a conserved glycine residue at position 100, whereas the *E. coli* and *Synechocystis* sp. strain PCC6803 homologs have nonconservative substitutions (data not shown). However, to determine the significance of this residue, we constructed a *H. influenzae* mutant (pKA4) that contains the *E. coli* *ispB* gene (Fig. 3). Strain KO229 harboring pKA4 showed similar growth characteristics to KO229 harboring pKA3 (Fig. 2). These results indicate that the *ispB* homolog from *H. influenzae* can complement the *ispB* disruptant.

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influenzae and Synechocystis sp. strain PCC6803, respectively (Fig. 2). The *H. influenzae* ispB homolog was suspected to encode an octaprenyl diphosphate synthase because of its high homology with *E. coli* ispB, but it is not known what type of demethyl-MK is synthesized in *H. influenzae* (3). The *Synechocystis* sp. strain PCC6803 ispB homolog was suspected to encode a solanesyl diphosphate synthase because of its high homology with *E. coli* ispB and its function in *H. influenzae* as a solanesyl diphosphate synthase because of its high homology with *E. coli* ispB and its function in *H. influenzae* as a solanesyl diphosphate synthase.

The *H. influenzae* ispB homolog was cloned into the expression vector pUC18 to obtain pMN18. The authenticity of both cloned genes was confirmed by sequence analysis by the dideoxy chain termination method (14) on an ABI prism 377 sequencer. No discrepancies with respect to the reported sequences were found. KO229 harboring pKA3 was transformed with either pMN18 or pSN18, and the resulting transformants were both spectinomycin and ampicillin resistant. The transformants were subcultured five times in L medium containing ampicillin at 50 μg/ml and plated on L agar medium containing ampicillin. The resulting colonies were then replicated on L agar medium containing ampicillin or spectinomycin. Spectinomycin-sensitive and ampicillin-resistant strains which had only pMN18 or pSN18, but not pKA3, were selected. The exchange of pKA3 for pMN18 or pSN18 was confirmed by Southern blot analysis of the plasmid DNA (data not shown). No KO229 strain cured of both plasmids was obtained. These results indicate that the *H. influenzae* and *Synechocystis* sp. strain PCC6803 ispB homologs can complement a defect in the *E. coli* ispB gene and confirm that chromosomal *ispB* gene disruptants are not viable unless they carry a plasmid-borne copy of this gene.

**FIG. 2. Alignment of the sequences of the ispB product from *E. coli* (EC) (GenBank accession no. U18897), its *H. influenzae* (HI) homolog HI0881 (GenBank accession no. U32770), and of *S. cerevisiae* COQ1 gene encoding hexaprenyl diphosphate synthase is not essential for fermentative growth but is required for respiratory growth (2, 11). We have recently reported that the *S. pombe* *dps* gene encoding decaprenyl diphosphate synthase is not essential for growth in rich medium but is essential for growth on minimal medium (16). Interestingly, this *dps* disruptant can grow on minimal medium when supplemented with cysteine, glutathione, or α-tocopherol, indicating that UQ functions as an antioxidant in yeast (16). These results suggest that different organisms may have distinct requirements for isoprenoid quinones during growth. Our results suggest that the *ispB* gene is more important for growth in *E. coli* than in yeasts.
tography with standard ubiquinone 10 carried out in a Kieselgel 60 F 254 plate. The extracted crude ubiquinone was analyzed by normal-phase thin-layer chromatography. This provides a notion of the precise function of a gene and that the gene (64.9%). This indicates that homology does not always define the length of the side chain of ubiquinone. Biochim. Biophys. Acta 169:217–223.


Further, our results show that various kinds of UQs can be produced in E. coli by simply expressing the corresponding polyprenyl diphosphate synthase from different organisms without any apparent effect on its growth properties. Our results also support previous findings that purB-hydroxybenzoate: octaprenyl diphosphate transferase (UbiA) has broad specificity with respect to its substrates (10, 17, 20). The UbiA protein could transfer not only the octaprenyl group but also the heptaprenyl and solanesyl groups to para-hydroxybenzoate.

In E. coli, the H. influenzae ispB homolog was found to produce UQ-7, although it was expected to encode octaprenyl diphosphate synthase on the basis of its high homology with this gene (64.9%). This indicates that homology does not always provide a notion of the precise function of a gene and that further experimental evidence is necessary to provide definite proof.

This work was supported by a Grant-in-Aid for special Scientific Research on Agriculture, Forestry and Fisheries.