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A Gene Cluster for the Mevalonate Pathway from *Streptomyces* sp. Strain CL190

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A biosynthetic 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.1.34), the rate-limiting enzyme of the mevalonate pathway for isopentenyl diphosphate biosynthesis, had previously been purified from *Streptomyces* sp. strain CL190 and its corresponding gene (*hmgr*) had been cloned (S. Takahashi, T. Kuzuyama, and H. Seto, J. Bacteriol. 181:1256–1263, 1999). Sequence analysis of the flanking regions of the *hmgr* gene revealed five new open reading frames, *orfA* to *-E*, which showed similarity to those encoding eucaryotic and archaebacterial enzymes for the mevalonate pathway. Feeding experiments with [1-^{13}C]acetate demonstrated that *Escherichia coli* JM109 harboring the *hmgr* gene and these open reading frames used the mevalonate pathway under induction with isopropyl β-D-thiogalactopyranoside. This transformant could grow in the presence of fosmidomycin, a potent and specific inhibitor of the nonmevalonate pathway, indicating that the mevalonate pathway, intrinsically absent in *E. coli*, is operating in the *E. coli* transformant. The *hmgr* gene and *orfABCDE* are thus unambiguously shown to be responsible for the mevalonate pathway and to form a gene cluster in the genome of *Streptomyces* sp. strain CL190.

Isoprenoids, found in all organisms, play important roles such as steroid hormones in mammals, carotenoids in plants, and ubiquinone or menaquinone in bacteria (28). All these isoprenoids are synthesized by consecutive condensations of the five-carbon monomer isopentenyl diphosphate (IPP). It was generally believed that IPP is synthesized only by condensation of three molecules of acetyl coenzyme A (CoA) through the mevalonate pathway (Fig. 1A). This ubiquitous pathway consists of six enzymes: acetoacetyl-CoA synthase, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase, and pyrophosphomevalonate decarboxylase.

There is an extensive body of information concerning the mevalonate pathway in eucaryotes including rats, mice, and yeast. However, very few studies on the enzymes or genes for this pathway in an additional kingdom, *Eubacteria*, are available. Recently, it has turned out that the mevalonate pathway is absent in many eubacteria including *Escherichia coli* and *Bacillus subtilis* (27). Instead of the mevalonate pathway, these eubacteria use the nonmevalonate pathway. The initial step of this newly identified pathway is the formation of 1-deoxy-D-xylulose 5-phosphate (DXP) by condensation of pyruvate and glyceraldehyde-3-phosphate catalyzed by DXP synthase (2, 14, 18, 20, 36). In the second step DXP is converted to 2-*C*-methyl-D-erythritol 4-phosphate (MEP) by DXP reductoisomerase as demonstrated by us previously (15, 16, 38). MEP is then cytidylylated by MEP cytidylyltransferase to give 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol (CDP-ME) (12, 26), which is phosphorylated by CDP-ME kinase to afford 2-phospho-4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol (CDP-ME2P) (13, 21). Recently we succeeded in cloning MEP cytidylyltransferase (12) and CDP-ME kinase (13) genes from *E. coli* and demonstrated unequivocally that these enzymes constitute the nonmevalonate pathway (Fig. 1B). The subsequent reactions leading to IPP from CDP-ME2P, however, remain unknown.

Feeding experiments with [^{14}C]acetyl-CoA, [^{14}C]HMG-CoA, and [^{14}C]mevalonate have demonstrated the involvement of the enzymes for the mevalonate pathway in some eubacteria such as *Myxococcus fulvus*, *Lactobacillus plantarum*, and *Staphylococcus carnosus* (7). Recent achievements in bacterial genome sequencing, however, revealed that all the eubacteria except for *Staphylococcus aureus* (http://www.sanger.ac.uk/Projects/S_aureus/) and the Lyme disease spirochaete *Borrelia burgdorferi* (5) utilized only the nonmevalonate pathway for isoprenoid biosynthesis, whereas four archaebacteria, *Methanococcus jannaschii* (3), *Methanobacterium thermoautotrophicum* (35), *Pyrococcus horikoshii* (10), and *Archaeoglobus fulgidus* (11), used only the mevalonate pathway. On the other hand, feeding experiments with ^{13}C-labeled acetate have proved that isoprenoids and hemiterpenoid metabolites such as naphterpin (31, 32), furaquinocin (6), napyradiomycin (34), and terpentecin (9) produced by the genus *Streptomyces* are synthesized through the mevalonate pathway. No genes encoding enzymes responsible for the mevalonate pathway, however, have been identified from these eubacteria except for the HMG-CoA reductase genes from naphterpin (37), furaquinocin (4), and terpentecin producers (4). Thus, our attention has been directed to the cloning of all other genes involved in the mevalonate pathway from naphterpin producer *Streptomyces* sp. strain CL190.

In this paper we describe the cloning and functional analyses of the five open reading frames (ORFs) newly found in the flanking regions of the HMG-CoA reductase gene (*hmgr*) from *Streptomyces* sp. strain CL190. A gene cluster containing the *hmgr* gene and these ORFs was heterogeneously expressed in *E. coli*, and a labeling pattern of ubiquinone from this *E. coli* transformant grown in the presence of [1-^{13}C]acetate was analyzed with nuclear magnetic resonance (NMR). This paper is

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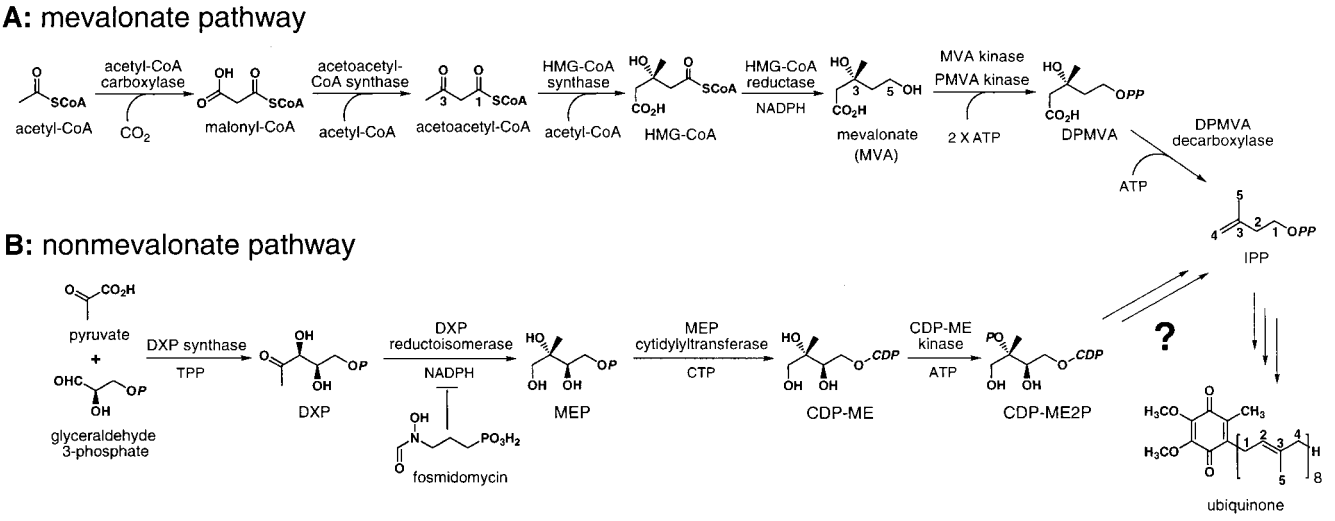

FIG. 1. Mevalonate and nonmevalonate pathways for IPP biosynthesis. Fosmidomycin is a potent and specific inhibitor of DXP reductoisomerase in the nonmevalonate pathway. The reactions leading to IPP from CDP-ME2P remain unknown. MVA, mevalonate; PMVA, phosphomevalonate; DPMVA, diphosphomevalonate; TPP, thiamine diphosphate.

the first to describe the functional assignment of eubacterial mevalonate pathway genes expressed in *E. coli*.

MATERIALS AND METHODS

Materials. [1-^{13}C]acetate (isotopic abundance, 99%) was purchased from ICON (New York, N.Y.). Fosmidomycin was a gift from Fujisawa Pharmaceutical Co., Ltd. All restriction enzymes, T4 DNA polymerase, and pUC118 were purchased from Takara Shuzo, Kyoto, Japan.

Bacterial strains and media. *Streptomyces* sp. strain CL190 was cultivated as described previously (33). *E. coli* JM109 was used as a cloning host and a host for the *lacZ* expression system. JM109 was grown in Luria-Bertani (LB) broth as described by Sambrook et al. (29). A minimum medium, M9 (29), which was used in the assay for fosmidomycin resistance, was always supplemented with 20 μg of thiamine/ml to meet the thiamine requirement of JM109.

Sequence analysis of the flanking regions of the *hmgr* gene. Total DNA from CL190, which was prepared as described previously (29), was partially digested with *Sau*3AI, followed by size fractionation by agarose gel electrophoresis. DNA fragments larger than 20 kb were ligated to a *Bam*HI- and phosphatase-treated pWE15 cosmid vector (Stratagene) to give a cosmid library of CL190. This cosmid library was screened by colony hybridization with a DNA fragment containing the CL190 *hmgr* gene (37) as a probe. Hybridization to this probe was found with 17 transformants. Seventeen cosmid clones, designated pCLC1 to pCLC17, were prepared from these positive transformants and digested with various restriction enzymes, and the positions of the hybridizing regions were defined by Southern hybridization (29) with the same probe. A series of plasmids, constructed by subcloning various hybridized DNA fragments into pUC118, were sequenced by a method described below.

Plasmid construction. A 6.7-kb fragment digested with *Sna*BI from cosmid pCLC13 was blunt ended with T4 DNA polymerase and then inserted into *Hin*cII- and alkaline phosphatase-treated pUC118 so that the ORFs were located in the same transcriptional direction as the *lacZ* promoter. *E. coli* JM109 was transformed with the resulting plasmid, pUMV19. This plasmid was digested with *Mlu*I, the recognition sites of which were in the targeted *orfD*, and then self-ligated to give deletion plasmid pUMV19ΔM (Fig. 2). Thus, this deletion plasmid lacked only *orfD*.

DNA sequence analysis. DNA sequences were determined by the dideoxy chain termination method (30) with an automated sequencer (model 4000L; Li-cor) and the protocol of the supplier. A FramePlot 2.3 program (8) was used for searching ORFs. The FASTA program (19, 23) performed a homology search of protein databases. Amino acid sequences aligned by the GENETYX program (Software Development, Tokyo, Japan) were then edited visually to align consensus motifs.

Purification of ubiquinone and its NMR analysis. *E. coli* JM109(pUMV19) was grown at 37°C in 1 liter of LB medium containing 0.1% sodium [1-^{13}C] acetate, 0.1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), and 50 μg of ampicillin/ml for 14 h. Ubiquinone (coenzyme Q8) was purified from this transformant as described previously (24) and was further purified by high-performance liquid chromatography with a PEGASIL octyldecyl silane column (4.6 by 250 mm; Senshu Scientific Co., Tokyo, Japan). Then purified ubiquinone was analyzed by ^{13}C-NMR (A-500; JEOL, Tokyo, Japan). As a control, ubiquinone purified from *E. coli* JM109(pUC118), which was grown under the same conditions as the transformant in the absence of [1-^{13}C]acetate, was also analyzed.

Determination of ^{13}C enrichment. Relative enrichments for all carbon atoms of the labeled ubiquinone were obtained by comparison of ^{13}C integrals with those of natural-abundance standards (1.1%). The intensities of two signals at 61 ppm in ^{13}C spectra for ubiquinone from both JM109 and JM109(pUMV19) were aligned. The signals were assigned to methoxy carbons in the ubiquinone molecule. These signals were utilized as standards for the evaluation of isotopic abundance.

Nucleotide sequence accession number. The nucleotide sequence of a 6.7-kb *Sna*BI-*Sna*BI fragment including the *hmgr* gene and *orfABCDE* has been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases with accession no. AB037666.

RESULTS AND DISCUSSION

DNA sequence analysis of the flanking regions of the *hmgr* genes. A 6.7-kb *Sna*BI-*Sna*BI DNA fragment including the flanking regions of the *hmgr* gene from *Streptomyces* sp. strain CL190 was sequenced. A FramePlot 2.3 program revealed the presence of five new ORFs, *orfA* to *-E*, in this DNA fragment. Organization of all ORFs in this 6.7-kb fragment is shown in Fig. 2. After a search of the SWISS-PROT database using a FASTA program, the products of *orfA*, *orfB*, *orfC*, and *orfE* were deduced to be mevalonate kinase (EC 2.7.1.36), diphosphomevalonate decarboxylase (EC 4.1.1.33), mevalonate kinase (EC 2.7.1.36), and HMG-CoA synthase (EC 4.1.3.5), respectively (Table 1). This search using the FASTA program, however, could not distinguish between the products of *orfA* and *orfC*; those of both ORFs showed homology with meval-

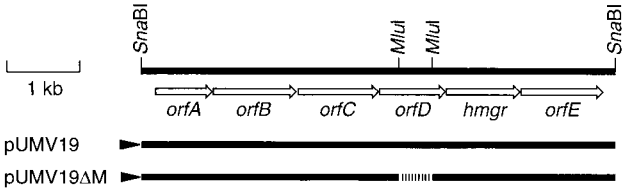

FIG. 2. Organization of ORFs in the 6.7-kb *Sna*BI-*Sna*BI fragment. The arrows indicate the extents and directions of the ORFs found in the flanking regions of the *hmgr* gene of *Streptomyces* sp. strain CL190. The arrowheads indicate the direction of the *lacZ* promoter in pUC118. pUMV19ΔM, derived from pUMV19, lacks only *orfD*.

TABLE 1. Deduced functions of *orfA* to *orfE*

ORF	No. of aa[a] encoded	Protein with the highest similarity (% identity/overlap [no. of aa])	Accession no.	Reference
orfA	345	Mevalonate kinase of *M. jannaschii* (26/306)	Q58487	3
orfB	350	Diphosphomevalonate decarboxylase of *S. cerevisiae* (34/319)	P32377	39
orfC	374	Mevalonate kinase of *Arabidopsis thaliana* (22/363)	P46086	25
orfD	363	Hypothetical protein of *E. herbicola* (33/334)	Q01335	1
orfE	389	HMG-CoA synthase of *A. thaliana* (28/371)	P54873	22

[a] aa, amino acids.

onate kinase. In addition to mevalonate kinase, the mevalonate pathway requires phosphomevalonate kinase (EC 2.7.4.2) for phosphorylation of phosphomevalonate (Fig. 1). To reveal which of the two ORFs encodes phosphomevalonate kinase, the amino acid sequences encoded by *orfA* and *orfC* were compared with those of phosphomevalonate kinases from *Saccharomyces cerevisiae* (accession no. P24521) and *Schizosaccharomyces pombe* (accession no. AL109739). This homology search using a GENETYX program revealed that the product of *orfC* showed significant homology with the *S. cerevisiae* (26.8% identity in 153 amino acids) and *S. pombe* (20.6% identity in 180 amino acids) enzymes. In addition, the product of *orfA* showed homology (24.9% identity in 217 amino acids) with mevalonate kinase from *S. pombe* (accession no. Q09780). The products of *orfC* and *orfA* were thus deduced to be phosphomevalonate kinase and mevalonate kinase, respectively.

The product of *orfD* showed significant homology with a hypothetical protein, ORF6, in *Erwinia herbicola* Eho10, which produced carotenoids (1). The associated gene, *orf6*, had been reported to be located in the carotenoid biosynthetic gene cluster in this organism. Since a frameshift mutation in the ORF did not affect carotenoid production (1), its biochemical function remained unclear. The function of *orfD* in the mevalonate pathway remains undefined in this study as well (see below).

NMR analyses of ubiquinone from an *E. coli* transformant harboring pUMV19. To elucidate the functions of these ORFs, the 6.7-kb *Sna*BI-*Sna*BI fragment was expressed by the *lacZ* system in *E. coli* and a feeding experiment with [1-^{13}C]acetate was carried out on this transformant. The ability of this transformant to utilize the mevalonate pathway was proved by this feeding experiment.

The ^{13}C-NMR spectrum of ubiquinone labeled with sodium [1-^{13}C]acetate showed enrichments at the C-1 (isotopic abundance, 1.6%) and C-3 (isotopic abundance, 4.6%) positions of eight prenyl units in the ubiquinone molecule (Fig. 3). These C-1 and C-3 carbons were derived from C-1 and C-3 of the IPP molecule, respectively (Fig. 1). On the other hand, no enrichments were found in the C-2, C-4, and C-5 positions of these prenyl units. The very large differential incorporation of the labeled acetate into mevalonate-derived ubiquinone needs to be explained, since to the best of our knowledge an unusual phenomenon such as this has never been reported. C-1 and C-3 of prenyl units of the ubiquinone side chain derive from C-1 and C-3, respectively, of acetoacetyl-CoA (Fig. 1), which unlike what occurs in the usual mevalonate pathway, is assumed to be formed by condensation of acetyl-CoA and malonyl-CoA in order to explain this unusual incorporation pattern. Since no gene of an acetoacetyl-CoA synthase (EC 2.3.1.9) from *Streptomyces* sp. strain CL190 was introduced in the transformant which intrinsically lacked the mevalonate pathway, this organism should have used its own acetoacetyl-CoA synthase from fatty acid biosynthesis for the formation of acetoacetyl-CoA. Interestingly, *E. coli* possesses two sequences of acetoacetyl-CoA synthase (accession no. P76461 and Q46936) showing 57.9% identity to each other in 392 amino acids. The roles of these enzymes in acetoacetyl-CoA synthesis, however, remain undefined. The labeling pattern described above clearly suggested that [1-^{13}C]acetate was efficiently incorporated into C-3 of mevalonate, corresponding to C-3 of IPP, via acetoacetyl-CoA. This labeled precursor was incorporated into C-5 of mevalonate, corresponding to C-1 of IPP, presumably with dilution by internal nonlabeled malonyl-CoA. An alternative explanation is to assume low activity of acetyl-CoA carboxylase to form malonyl-CoA from acetyl-CoA in *E. coli*. Further experiments are needed to uncover this unusual phenomenon. In any case, since two ^{13}C atoms from two molecules of [1-^{13}C]acetate incorporated through the mevalonate pathway label C-1 and C-3 of the IPP molecule, the labeling patterns of ubiquinone in this experiment thus proved the operation of the mevalonate pathway in *E. coli* JM109(pUMV19).

An *E. coli* transformant harboring pUMV19 was resistant to fosmidomycin. We next investigated whether *E. coli* JM109 (pUMV19) could grow in the presence of fosmidomycin, a potent and specific inhibitor of DXP reductoisomerase in the

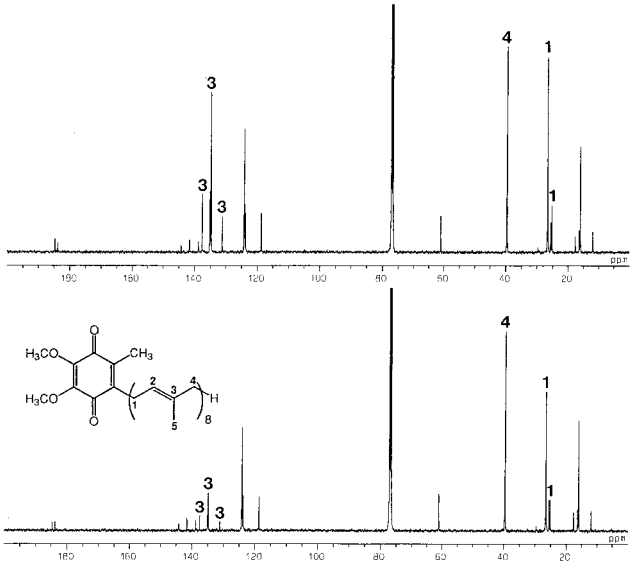

FIG. 3. ^{13}C-NMR spectra of ubiquinone from JM109 and the transformant harboring pUMV19. Ubiquinone was purified from JM109 (lower), and its transformant harboring pUMV19 was grown in LB medium containing 0.1% sodium [1-^{13}C]acetate under induction with 0.1 mM IPTG. The intensities of two signals at 61 ppm in both ^{13}C-NMR spectra were aligned. These signals were utilized as standards for the evaluation of isotopic abundance.

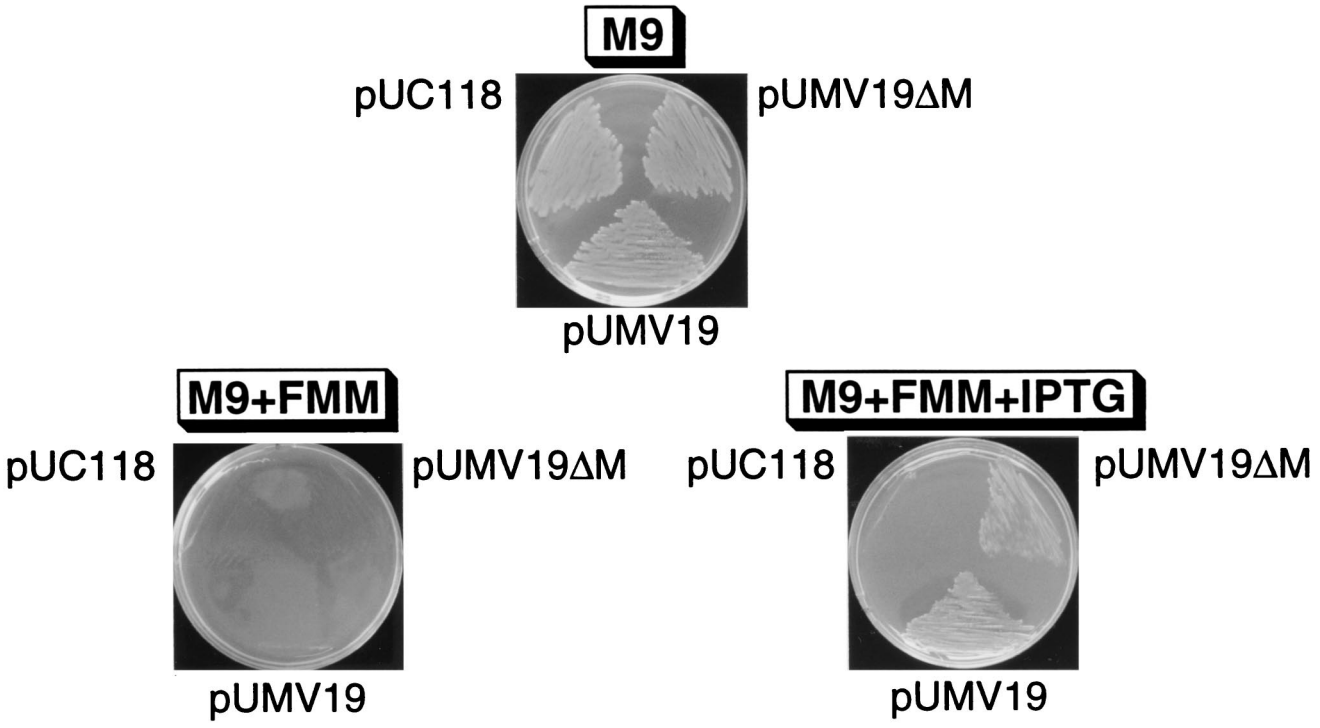

FIG. 4. Phenotypes of JM109 and the transformants. All plates are shown after overnight incubation at 37°C. Minimum medium M9 used in this experiment was always supplemented with 20 μg of thiamine/ml to meet the thiamine requirement of *E. coli* JM109. JM109, JM109(pUMV19), and JM109(pUMV19ΔM) could not grow on the M9 plate containing 20 μg of fosmidomycin (FMM)/ml (lower left), but JM109(pUMV19) and JM109(pUMV19ΔM) could grow on the plate under induction with 0.1 mM IPTG (lower right). On the lower-right plate, the growth of JM109(pUMV19ΔM) was slower than that of JM109(pUMV19).

nonmevalonate pathway (17). If this transformant could utilize the mevalonate pathway for IPP biosynthesis, it should grow in the presence of this inhibitor. *E. coli* JM109(pUC118) did not grow on minimum agar medium M9 containing 20 μg of fosmidomycin/ml (lower left and lower right in Fig. 4). On the other hand, *E. coli* JM109(pUMV19) could grow on the M9 medium containing fosmidomycin when there was induction with IPTG (lower right in Fig. 4). These results unambiguously established that *E. coli* JM109(pUMV19) harboring *orfA* to *-E* utilized the mevalonate pathway for IPP biosynthesis. *orfA* to *-E* have thus been proved to supply IPP in *E. coli*. The role of *orfD*, however, remains to be clarified.

***orfD* is necessary for full operation of the mevalonate pathway.** In order to gain insight into the function of *orfD*, pUMV19ΔM, lacking only *orfD*, was constructed from pUMV19. *E. coli* JM109 was transformed with pUMV19ΔM, and then the ability of the resulting transformant to grow in the presence of fosmidomycin was investigated. This transformant could grow on the M9 medium plate containing fosmidomycin only under induction with IPTG, but its growth seemed to be slower than that of *E. coli* JM109(pUMV19) (Fig. 4, lower right). The same phenomenon was also observed in a liquid M9 medium containing fosmidomycin; *E. coli* JM109(pUMV19) grew to an optical density at 660 nm of 2.5 (full growth), whereas *E. coli* JM109(pUMV19ΔM) grew to an optical density of 1.0 or less. These results indicated that *orfD* was not essential for operation in *E. coli* of the mevalonate pathway but was necessary for full operation of the pathway. A gene cluster containing *orfABCE* and the *hmgr* gene is thus shown to be essential for the operation of the mevalonate pathway in *E. coli*. Since wild-type *E. coli* has acetyl-CoA carboxylase and acetoacetyl-CoA synthase genes in the genome, it is not necessary to introduce these enzyme genes for the operation of the mevalonate pathway in *E. coli*.

Conclusion. In conclusion, it has been suggested that *orfA*, *orfB*, *orfC*, and *orfE* encode mevalonate kinase, diphosphomevalonate decarboxylase, phosphomevalonate kinase, and HMG-CoA synthase, respectively, and that they form a gene cluster together with the *hmgr* gene in the genome of *Streptomyces* sp. strain CL190. We demonstrated in this study that the *E. coli* transformant harboring this gene cluster could utilize the mevalonate pathway for IPP biosynthesis in addition to the nonmevalonate pathway. The transformant has proved to be a useful strain for preparing mutants possessing a metabolic block(s) in the nonmevalonate pathway (12, 13), because the associated mutations could be complemented by the expression of the *hmgr* gene and *orfABCDE* cloned in this study.

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M. Takagi and T. Kuzuyama contributed equally to this work.

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REFERENCES

1. **Armstrong, G. A., D. H. Burke, and J. E. Hearst.** 1994. Functional assignment of *Erwinia herbicola* Eho10 carotenoid genes expressed in *Escherichia coli*. Mol. Gen. Genet. **245:**406–416.
2. **Bouvier, F., A. d'Harlingue, C. Suire, R. A. Backhous, and B. Camara.** 1998. Dedicated roles of plastid transketolases during the early onset of isoprenoid biogenesis in pepper fruits. Plant Physiol. **117:**1423–1431.

3. **Bult, C. J., O. White, G. J. Olsen, L. Zhou, R. D. Fleischmann, G. G. Sutton, J. A. Blake, L. M. FitzGerald, R. A. Clayton, J. D. Gocayne, A. R. Kerlavage, M. A. Dougherty, J. F. Tomb, M. D. Adams, C. I. Reich, R. Overbeek, E. F. Kirkness, K. G. Weinstock, J. M. Merrick, A. Glodek, J. L. Scott, N. S. M. Geoghagen, and J. C. Venter.** 1996. Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. Science **273:**1058–1073.
4. **Dairi, T., Y. Motohira, T. Kuzuyama, S. Takahashi, N. Itoh, and H. Seto.** 2000. Cloning of the gene encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase from terpenoid antibiotics-producing *Streptomyces* strains. Mol. Gen. Genet. **262:**957–964.
5. **Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. C. Venter, et al.** 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature **390:** 580–586.
6. **Funayama, S., M. Ishibashi, K. Komiyama, and S. Omura.** 1990. Biosynthesis of furaquinocins A and B. J. Org. Chem. **55:**1132–1133.
7. **Horbach, S., H. Sahm, and R. Welle.** 1993. Isoprenoid biosynthesis in bacteria: two different pathways? FEMS Microbiol. Lett. **111:**135–140.
8. **Ishikawa, J., and K. Hotta.** 1999. FramePlot: a new implementation of the frame analysis for predicting protein-coding regions in bacterial DNA with a high G + C content. FEMS Microbiol. Lett. **174:**251–253.
9. **Isshiki, K., T. Tamamura, T. Sawa, H. Naganawa, T. Takeuchi, and H. Umezawa.** 1986. Biosynthetic studies of terpentecin. J. Antibiot. **39:**1634–1635.
10. **Kawarabayasi, Y., M. Sawada, H. Horikawa, Y. Haikawa, Y. Hino, S. Yamamoto, M. Sekine, S. Baba, H. Kosugi, A. Hosoyama, Y. Nagai, M. Sakai, K. Ogura, R. Otsuka, H. Nakazawa, M. Takamiya, Y. Ohfuku, T. Funahashi, T. Tanaka, Y. Kudoh, J. Yamazaki, N. Kushida, A. Oguchi, K. Aoki, and H. Kikuchi.** 1998. Complete sequence and gene organization of the genome of a hyper-thermophilic archaebacterium, *Pyrococcus horikoshii* OT3. DNA Res. **5:**55–76.
11. **Klenk, H. P., R. A. Clayton, J. F. Tomb, O. White, K. E. Nelson, K. A. Ketchum, R. J. Dodson, M. Gwinn, E. K. Hickey, J. D. Peterson, D. L. Richardson, A. R. Kerlavage, D. E. Graham, N. C. Kyrpides, R. D. Fleischmann, J. Quackenbush, N. H. Lee, G. G. Sutton, S. Gill, E. F. Kirkness, B. A. Dougherty, K. McKenney, M. D. Adams, B. Loftus, J. C. Venter, et al.** 1997. The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature **390:**364–370.
12. **Kuzuyama, T., M. Takagi, K. Kaneda, T. Dairi, and H. Seto.** 2000. Formation of 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol from 2-*C*-methyl-D-erythritol 4-phosphate by 2-*C*-methyl-D-erythritol 4-phosphate cytidylyltransferase, a new enzyme in the nonmevalonate pathway. Tetrahedron Lett. **41:**703–706.
13. **Kuzuyama, T., M. Takagi, K. Kaneda, H. Watanabe, T. Dairi, and H. Seto.** 2000. Studies on the nonmevalonate pathway: conversion of 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol to its 2-phospho derivative by 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol kinase. Tetrahedron Lett. **41:** 2925–2928.
14. **Kuzuyama, T., M. Takagi, S. Takahashi, and H. Seto.** 2000. Cloning and characterization of 1-deoxy-D-xylulose 5-phosphate synthase from *Streptomyces* sp. strain CL190, which uses both the mevalonate and nonmevalonate pathways for isopentenyl diphosphate biosynthesis. J. Bacteriol. **182:**891–897.
15. **Kuzuyama, T., S. Takahashi, H. Watanabe, and H. Seto.** 1998. Direct formation of 2-*C*-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate by 1-deoxy-D-xylulose 5-phosphate reductoisomerase, a new enzyme in the non-mevalonate pathway to isopentenyl diphosphate. Tetrahedron Lett. **39:**4509–4512.
16. **Kuzuyama, T., S. Takahashi, and H. Seto.** 1999. Construction and characterization of *Escherichia coli* disruptants defective in the *yaeM* gene. Biosci. Biotechnol. Biochem. **63:**776–778.
17. **Kuzuyama, T., T. Shimizu, S. Takahashi, and H. Seto.** 1998. Fosmidomycin, a specific inhibitor of 1-deoxy-D-xylulose 5-phosphate reductoisomerase in the nonmevalonate pathway for terpenoid biosynthesis. Tetrahedron Lett. **39:**7913–7916.
18. **Lange, B. M., M. R. Wildung, D. McCaskill, and R. Croteau.** 1998. A family of transketolases that directs isoprenoid biosynthesis via a mevalonate-independent pathway. Proc. Natl. Acad. Sci. USA **95:**2100–2104.
19. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive similarity protein searches. Science **227:**1435–1441.
20. **Lois, L. M., N. Campos, S. R. Putra, K. Danielsen, M. Rohmer, and A. Boronat.** 1998. Cloning and characterization of a gene from *Escherichia coli* encoding a transketolase-like enzyme that catalyzes the synthesis of D-1-deoxylylulose 5-phosphate, a common precursor for isoprenoid, thiamine, and pyridoxol biosynthesis. Proc. Natl. Acad. Sci. USA **95:**2105–2110.
21. **Lüttgen, H., F. Rohdich, S. Herz, J. Wungsintaweekul, S. Hecht, C. A. Schuhr, M. Fellermeier, S. Sagner, M. H. Zenk, A. Bacher, and E. Eisenreich.** 2000. Biosynthesis of terpenoids: YchB protein of *Escherichia coli* phosphorylates the 2-hydroxy group of 4-diphosphocytidyl-2*C*-methyl-D-erythritol. Proc. Natl. Acad. Sci. USA **97:**1062–1067.
22. **Montamat, F., M. Guilloton, F. Karst, S. Delrot.** 1995. Isolation and characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl-coenzyme A synthase. Gene **167:**197–201.
23. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
24. **Putra, S. R., A. Disch, J. M. Bravo, and M. Rohmer.** 1998. Distribution of mevalonate and glyceraldehyde 3-phosphate/pyruvate routes for isoprenoid biosynthesis in some gram-negative bacteria and mycobacteria. FEMS Microbiol. Lett. **164:**169–175.
25. **Riou, C., Y. Tourte, F. Lacroute, and F. Karst.** 1994. Isolation and characterization of a cDNA encoding *Arabidopsis thaliana* mevalonate kinase by genetic complementation in yeast. Gene **148:**293–297.
26. **Rohdich, F., J. Wungsintaweekul, M. Fellermeier, S. Sagner, S. Herz, K. Kis, W. Eisenreich, A. Bacher, and M. H. Zenk.** 1999. Cytidine 5′-triphosphate-dependent biosynthesis of isoprenoids: YgbP protein of *Escherichia coli* catalyzes the formation of 4-diphosphocytidyl-2-*C*-methylerythritol. Proc. Natl. Acad. Sci. USA **96:**11758–11763.
27. **Rohmer, M.** 1999. Isoprenoids including carotenoids and steroids, p. 45–67. *In* D. Barton and K. Nakanishi (ed.), Comprehensive natural products chemistry, vol. 2. Elsevier, Amsterdam, The Netherlands.
28. **Sacchettini, J. C., and C. D. Poulter.** 1997. Creating isoprenoid diversity. Science **277:**1788–1789.
29. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
31. **Seto, H., H. Watanabe, and K. Furihata.** 1996. Simultaneous operation of the mevalonate and non-mevalonate pathways in the biosynthesis of isopentenyl diphosphate in *Streptomyces aeriouvifer*. Tetrahedron Lett. **37:**7979–7982.
32. **Shin-ya, K., K. Furihata, Y. Hayakawa, and H. Seto.** 1990. Biosynthetic studies of naphterpin, a terpenoid metabolite of *Streptomyces*. Tetrahedron Lett. **31:**6025–6026.
33. **Shin-ya, K., S. Imai, K. Furihata, Y. Hayakawa, Y. Kato, G. D. Vanduyne, J. Clardy, and H. Seto.** 1990. Isolation and structural elucidation of an antioxidative agent, naphterpin. J. Antibiot. **43:**444–447.
34. **Shiomi, K., H. Iinuma, H. Naganawa, K. Isshiki, T. Takeuchi, and H. Umezawa.** 1987. Biosynthesis of napyradiomycins. J. Antibiot. **40:**1740–1745.
35. **Smith, D. R., L. A. Doucette-Stamm, C. Deloughery, H. Lee, J. Dubois, T. Aldredge, R. Bashirzadeh, D. Blakely, R. Cook, K. Gilbert, D. Harrison, L. Hoang, P. Keagle, W. Lumm, B. Pothier, D. Qiu, R. Spadafora, R. Vicaire, Y. Wang, J. Wierzbowski, R. Gibson, N. Jiwani, A. Caruso, D. Bush, H. Safer, D. Patwell, S. Prabhakar, S. McDougall, G. Shimer, A. Goyal, S. Pietrokovski, G. M. Church, C. J. Daniels, J.-I. Mao, P. Rice, J. Nölling, and J. N. Reeve.** 1997. Complete genome sequence of *Methanobacterium thermoautotrophicum* ΔH: functional analysis and comparative genomics. J. Bacteriol. **179:**7135–7155.
36. **Sprenger, G. A., U. Schorken, T. Wiegert, S. Grolle, A. A. Graaf, S. V. Taylar, T. P. Begley, S. Bringer-Meyer, and H. Sahm.** 1997. Identification of a thiamin-dependent synthase in *Escherichia coli* required for the formation of the 1-deoxy-D-xylulose 5-phosphate precursor to isoprenoids, thiamin, and pyridoxol. Proc. Natl. Acad. Sci. USA **94:**12857–12862.
37. **Takahashi, S., T. Kuzuyama, and H. Seto.** 1999. Purification, characterization, and cloning of a eubacterial 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key enzyme involved in biosynthesis of terpenoids. J. Bacteriol. **181:**1256–1263.
38. **Takahashi, S., T. Kuzuyama, H. Watanabe, and H. Seto.** 1998. A 1-deoxy-D-xylulose 5-phosphate reductoisomerase catalyzing the formation of 2-*C*-methyl-D-erythritol 4-phosphate in an alternative nonmevalonate pathway for terpenoid biosynthesis. Proc. Natl. Acad. Sci. USA **95:**9879–9884.
39. **Toth, M. J., and L. Huwyler.** 1996. Molecular cloning and expression of the cDNAs encoding human and yeast mevalonate pyrophosphate decarboxylase. J. Biol. Chem. **271:**7895–7898.