Methanocaldococcus jannaschii Uses a Modified Mevalonate Pathway for Biosynthesis of Isopentenyl Diphosphate

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Archaea have been shown to produce isoprenoids from mevalonate; however, genome analysis has failed to identify several genes in the mevalonate pathway on the basis of sequence similarity. A predicted archaeal kinase, coded for by the MJ0044 gene, was associated with other mevalonate pathway genes in the archaea and was predicted to be the "missing" phosphomevalonate kinase. The MJ0044-derived protein was tested for phosphomevalonate kinase activity and was found not to catalyze this reaction. The MJ0044 gene product was found to phosphorylate isopentenyl phosphate, generating isopentenyl diphosphate. Unlike other known kinases associated with isoprene biosynthesis, *Methanocaldococcus jannaschii* isopentenyl phosphate kinase is predicted to be a member of the aspartokinase superfamily.

Isoprenoids are a large family of natural products that includes metabolically and medically important compounds such as cholesterol, steroid hormones, ubiquinone, carotenoids, and taxol. In archaea, isoprenoids are of particular interest because they are the major component of their membrane lipids (22). Archaeal lipids are composed of isoprenoid side chains connected to *sn*-glycerol-1-phosphate through ether linkages. Thus, the archaeal lipids differ from the majority of eukaryotic and eubacterial lipids in the isoprene nature of the alkyl chain, the stereoconfiguration of the glycerol moiety, and the presence of ether rather than ester linkages (22).

Despite the structural diversity seen in this group of compounds, all isoprenoids are derived from two precursor compounds: isopentenyl diphosphate (IPP) and its isomer 3,3-dimethylallyl diphosphate. Two pathways for the biosynthesis of these central metabolites are currently known: the mevalonate and the deoxy-D-xylulose 5-phosphate (DXP) pathways (19). The DXP pathway is known to function in the majority of bacteria and plant plastids, while the mevalonate pathway is typically found in animals, plant cytosol, and archaea. Some bacteria, along with plants, have been shown to operate with both pathways (19).

Labeled precursor studies have shown that both acetate and mevalonate are precursors for isoprenoids in archaea (13, 14, 33). These observations were supported through archaeal genome analysis which revealed homologs of mevalonate pathway enzymes, including 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) synthase and HMG-CoA reductase (9, 20, 31). Putative genes for other enzymes in the mevalonate pathway, however, were not immediately identified on the basis of sequence similarity. However, two additional enzymes of the pathway, mevalonate kinase and IPP isomerase, were subsequently identified and characterized (4, 17).

Although *Sulfolobus tokodaii* and *Sulfolobus solfataricus* have homologs for all mevalonate pathway enzymes, genomic analysis was unable to identify genes for phosphomevalonate kinase or diphosphomevalonate decarboxylase in most archaea (7). Similarly, several species of *Halobacteria* have gene homologs for diphosphomevalonate decarboxylase but not for phosphomevalonate kinase (7). Phosphomevalonate kinase and diphosphomevalonate decarboxylase catalyze two of the central steps in the formation of IPP from mevalonate, making the absence of homologs in most archaea intriguing. Two possible explanations for the apparent absence of these genes exist. First, these steps may be catalyzed by nonorthologous enzymes, making identification based on sequence similarity impossible. An alternative explanation is that the archaea may utilize an alternate route to IPP from phosphomevalonate, thus requiring different enzymes.

Smit and Mushegian have attempted to identify the missing isoprene biosynthetic enzymes by analyzing protein superfamilies that catalyze similar reactions and have proposed some candidate genes (32). This analysis allowed for the identification of an archaeal kinase in *Methanocaldococcus jannaschii*, MJ0044, which is proximal to genes for other mevalonate pathway enzymes in many archaea, including *Methanothermobacter thermoautotrophicum*, *Archaeoglobus fulgidus*, and *Methanosarcina mazei* (Fig. 1).

We reanalyzed archaeal genomes for genes that were chromosomally colocalized with mevalonate pathway genes and initially agreed with Smit et al. that the MJ0044 gene product could be the "missing" archaeal phosphomevalonate kinase. Here we report the characterization of the MJ0044 gene product and show that this kinase is not a mevalonate phosphate kinase but rather catalyzed the formation of IPP from isopentenyl phosphate (IP).

MATERIALS AND METHODS

Chemicals. Mevalonate was prepared by hydrolysis of mevalonate lactone in base as described by Huang et al. (17). Mevalonate phosphate was a gift from Henry M. Miziorko, Medical College of Wisconsin (35). Isopentenyl phosphate was synthesized as described below. All other chemicals and reagents were obtained from Sigma-Aldrich.

Synthesis of isopentenyl phosphate. Isopentenyl phosphate was prepared by coupling 2-cyanoethyl phosphate and 3-methyl-3-buten-1-ol followed by mild alkaline hydrolysis according to the method of Tener (34). Barium 2-cyanoethyl

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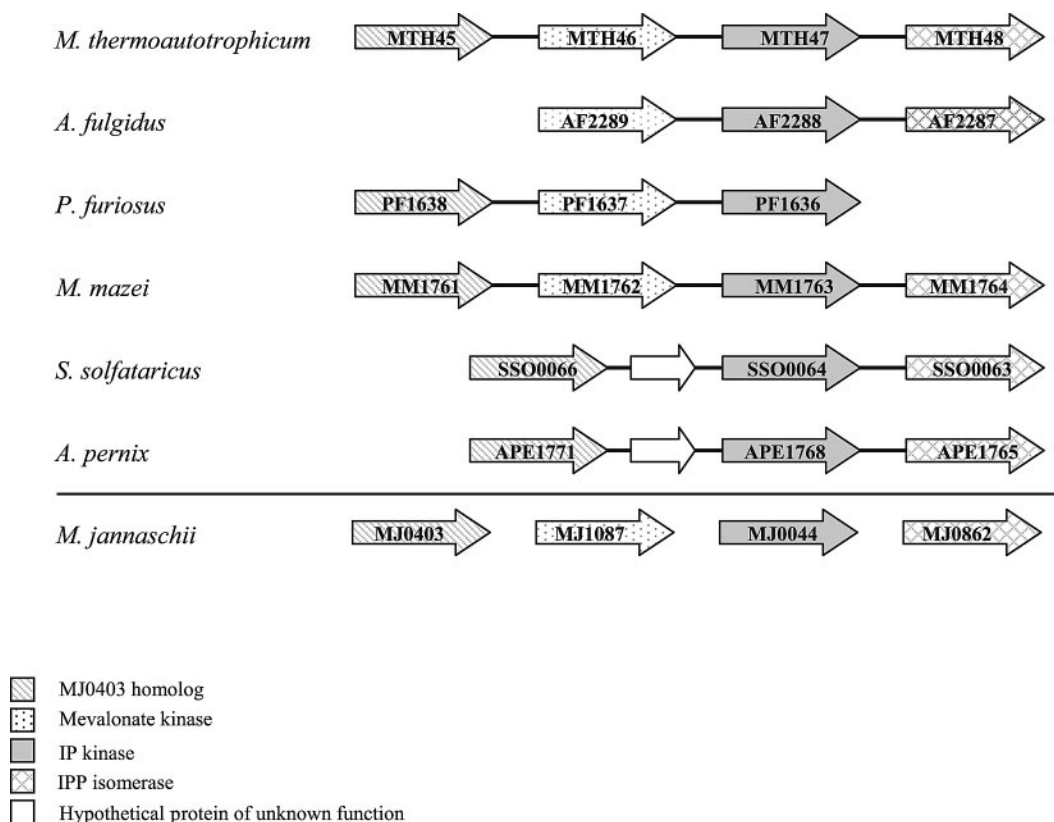
FIG. 1. Chromosomal organization of genes coding for mevalonate pathway enzymes in representative archaea, including *Methanothermobacter thermoautotrophicum*, *Archaeoglobus fulgidus*, *Pyrococcus furiosus*, *Methanosarcina mazei*, *Sulfolobus solfataricus*, and *Aeropyrum pernix*. The corresponding genes from *M. jannaschii* are indicated for comparison but are not colocalized on the chromosome. Open reading frame lengths are not drawn to scale.

phosphate (1 mmol, 322 mg) was suspended in 1 ml of water and mixed with 2 ml of a suspension of Dowex 50W-X8 H$^+$ in water. The resulting slurry was poured into a column, and the 2-cyanoethyl phosphate was eluted with 1 ml water. The combined eluate was dried by evaporation of the water, dissolved in pyridine (1 ml), and evaporated from pyridine (1 ml) two additional times before finally being dissolved in 1.5 ml of pyridine. To this solution was added 100 μl of 3-methyl-3-buten-1-ol (1 mmol) followed by 660 mg of dicyclohexylcarbodiimide, and the mixture was stirred for 2 days at room temperature. After the addition of 0.5 ml of water and stirring for 30 min, an additional 5 ml of water was added and the sample was filtered to remove the 1,3-dicyclohexylurea precipitate. The resulting sample was concentrated by evaporation with nitrogen, and the 2-cyanoethyl phosphate derivative of 3-methyl-3-buten-1-ol was extracted twice with 2 ml methylene chloride. The combined extracts were dried over Na$_2$SO$_4$ and evaporated. The yield of crude 2-cyanoethyl derivative was 0.25 g. The sample was dissolved in 8 ml of 1 M aqueous cyclohexylamine, held for 12 h at 3°C, and concentrated by evaporation of the solvent with a stream of nitrogen gas, and the excess cyclohexylamine was extracted three times with 2 ml methylene chloride. The sample was concentrated and the cyclohexylamine salt crystallized from either ethanol or ethanol-water mixtures to yield 124 mg of white crystals.

^{1}H nuclear magnetic resonance (NMR) (400 MHz; D$_2$O) of the isopentenyl phosphate didicyclohexylamine salt showed chemical shifts, referenced to sodium 3-trimethylsilylpropionate-2,2,3,3-d$_4$ (TMS), at the following values: IP δ, 4.844 (2H, m, vinyl hydrogens), 3.883 (2H, dt, $J_{H-1\rightarrow P}$ = 5.66 Hz, $J_{H-1\rightarrow H-2,2'}$ = 7.03 Hz, C-1 methylene), 2.350 (2H, t, $J_{H-2\rightarrow H-1,1'}$ = 7.03 Hz, C-2 methylene), and 1.780 (3H, s, 3-methyl). In addition, resonance values of δ 3.16 (2H, m, H-C-NH$_3$$^+$), 2.0 (4H, m, NH$_3$$^+$-CH-C$H_{eq}$), 1.82 (4H, m, NH$_3$$^+$-CH-C$H_{ax}$), and 1.35 (12H, m, CH$_2$) for two equivalents of the dicyclohexylamine salt were also observed. ^{13}C NMR (D$_2$O) showed chemical shifts at δ 113.9 (CH$_2$=), 65.7 (CH$_2$OP), 33.2 (CH$_2$), and 24.7 (3-methyl) for the isopentenyl phosphate and δ 53.0 (C-1), 33.2 (C-2 and C-2'), 27.0 (C-4), and 26.6 (C-3 and C-3') for the cyclohexylamine salt. Proton-decoupled ^{31}P NMR showed a single resonance at δ 4.38 (1P, s). Negative matrix-assisted laser desorption ionization (MALDI)

analysis performed using a 2,5-hydroxybenzoic acid matrix showed a [M-H]$^-$ ion at *m/z* 165. A solution containing 1 mg of the sample in water (100 μl) and passed through a Dowex 50W-X8 H$^+$ column with subsequent removal of the water by evaporation produced the free acid of isopentenyl phosphate. Reaction of this product with 20 μl of a mixture of pyridine, hexamethyldisilazane, and chlorotrimethylsilane (9:3:1 [vol/vol/vol]) for 2 min at 100°C followed by electron impact mass spectral analysis showed M$^+$ = 310 *m/z*, M$^+$-15 (CH$_3$) = 295 *m/z*, M$^+$-67 = 243 *m/z*, M$^+$-15 -68 (CH$_2$=C(CH$_3$)-CH=CH$_2$) = 227 *m/z*, and M$^+$-15 -84 (CH$_2$=C(CH$_3$)-CH$_2$-CH=O) = 211 *m/z* for the di-TMS derivative.

Cloning and heterologous expression of MJ0044. The *M. jannaschii* gene MJ0044 (Swiss-Prot accession number Q60352) was amplified by PCR from genomic DNA by use of the following oligonucleotide primers synthesized by Invitrogen: for MJ0044-F, 5′-GGTCATATGCTAACCATATTAAAATTAGG-3′, and for MJ0044-R, 5′-GCTGGATCCTTATTCTGAAAAATC-3′. PCR was performed as described previously using a 55°C annealing temperature (15). The amplified PCR product was purified by use of QIAquick spin column (QIAGEN), digested with restriction enzymes NdeI and BamHI, and then ligated into the compatible sites in plasmid pT7-7 (USB). DNA sequences were verified by dye-terminator sequencing at the Virginia Bioinformatics Institute's DNA facility. The resulting plasmid, pMJ0044, was transformed into *Escherichia coli* BL21-CodonPlus (DE3)-RIL cells (Stratagene). The transformed cells were grown in Luria-Bertani medium (Difco) (200 ml) supplemented with 100 μg/ml ampicillin at 37°C with shaking until they reached an optical density at 600 nm of 1.0. Recombinant protein production was induced by addition of lactose to a final concentration of 28 mM. After an additional 2 h of culture, the cells were harvested by centrifugation (4,000 × *g*, 5 min) and frozen at −20°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of total cellular proteins by use of a Tris-glycine buffer system confirmed induction of the desired protein. *E. coli* cells expressing recombinant protein were resuspended in 4 ml extraction buffer [50 mM *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (pH 7.0), 10 mM MgCl$_2$, 20 mM dithiothreitol] and lysed by sonication. After the majority of *E. coli* proteins was precipitated by heating of the cell lysate

to 80°C for 10 min, the MJ0044-derived protein was purified by anion exchange chromatography on a MonoQ HR column (Amersham Bioscience) (1 by 8 cm) with a linear gradient of 0 to 1 M NaCl in 25 mM Tris (pH 7.5) (over 55 ml) at 1 ml/min. The resulting MJ0044-derived protein ran as a single band at approximately 30 kDa and was >98% pure, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with Coomassie blue staining. Protein concentration was determined by Bradford analysis (8).

Enzymatic analysis of MJ0044 gene product. Activity of IP kinase was determined by measuring the generation of ADP through a coupled assay with pyruvate kinase and lactate dehydrogenase (1). An initial continuous assay run at room temperature included 7 units pyruvate kinase, 8 units lactate dehydrogenase, 50 mM Tris buffer (pH 7.5), 4 mM ATP, 0.8 mM IP, 7 mM MgCl$_2$, 3 mM 2-mercaptoethanol, 5 mM phosphoenolpyruvate, and 0.25 mM NADH in a final volume of 1 ml. The reaction was initiated with the addition of IP kinase (0.56 µg), and an initial linear decrease in absorbance at 340 nm was observed. The reaction was allowed to continue until complete consumption of NADH occurred (~30 min). A discontinuous assay was used in subsequent experiments to allow for incubation of IP kinase reactions at the higher operating temperatures of the thermophilic MJ0044-derived protein. IP kinase (0.14 µg) was incubated for 20 min at 55°C in a reaction mixture containing 4 mM ATP, 0.8 mM IP, 7 mM MgCl$_2$, 3 mM 2-mercaptoethanol, and 150 mM Tris buffer (pH 7.5) in a final volume of 1 ml. Following incubation, samples were cooled on ice. For measurement of ADP in the reaction mixture, 200 to 1,000 µl of the reaction mixture was transferred to a cuvette containing water to give a final volume of 1,000 µl. Phosphoenolpyruvate and NADH were added to concentrations of 5 mM and 0.25 mM, respectively. Enzyme activity was measured at room temperature by monitoring the total decrease in absorbance at 340 nm following the addition of 7 units pyruvate kinase and 8 units lactate dehydrogenase. Replicate analysis of individual incubation mixtures following extended incubation on ice showed that there was no significant change in the amount of ADP detected over time. This indicated that IP kinase activity was minimal at 0°C. Although ADP is the preferred nucleotide substrate for pyruvate kinase, it also utilizes a number of alternate nucleotides, including GDP (5, 29), thus allowing us to determine whether IP kinase could utilize GTP in place of ATP. For kinetic assays, the IP concentration was adjusted from 0.01 mM to 2 mM. Kinetic parameters were estimated from the slope and intercepts of the Lineweaver-Burk plot using Microsoft Excel software.

The activity of IP kinase was determined in 0.5 pH increments between pH 5 and pH 10 by use of a three-component buffer system (28) consisting of 15 mM Bis-Tris, 7.6 mM N-2-hydroxyethylpiperazine-N′-3-propanesulfonic acid, and 7.6 mM 2-[N-cyclohexylamino]ethanesulfonic acid in place of Tris buffer used in the standard assays. Following incubation at 55°C for 20 min, 250 µl of the incubation mixture was combined with 500 µl 0.5 M Tris buffer (pH 7.5)–250 µl H$_2$O–50 µl 0.1 M phosphoenolpyruvate–2.5 µl 0.1 M NADH. The total decrease in absorbance at 340 nm was measured following the addition of 7 units pyruvate kinase and 8 units lactate dehydrogenase.

Analysis of enzymatically generated reaction product. For mass spectral determination of IP kinase products, 4 mM ATP, 4 mM MgCl$_2$, and 1 mM 2-mercaptoethanol were combined in 870 µl water and was adjusted to ~7.0 with 1 M NaOH. The solution was made 1.5 mM with IP, 0.14 µg of enzyme was added to a final volume of 1 ml, and the mixture was incubated for 1 h at 55°C. The control reaction contained no enzyme. Following incubation the samples were processed by two different methods for mass spectral analysis. One involved the analysis of the TMS derivatives of the reaction mixtures and the other MALDI analysis. Negative-ion MALDI analysis of 0.1 µl of 10× concentrated reaction mixture, by use of a 2,5-hydroxybenzoic acid matrix, showed an [M-H]⁻ ion at m/z 245, corresponding to IPP, from the sample but not from the control.

For the analysis of the TMS derivatives, each sample was passed through a Dowex 50W-X8 pyridinium column, concentrated to dryness by evaporation with nitrogen gas, and then reacted with the TMS reagent (pyridine, hexamethyldisilazane, and chlorotrimethylsilane) (9:3:1 [vol/vol/vol]) for 10 min at 100°C. Mass spectral analysis (70 eV) of samples converted into the TMS derivative by gas chromatography-mass spectrometry and direct injection showed that only the sample incubated with enzyme contained pyrophosphate. This was confirmed by the measurement of M⁺ = 466 and M⁺-15 = 451 m/z for the tetra-TMS derivative, which was identical to a known TMS derivative of pyrophosphate. The pyrophosphate was observed with samples but not with the controls by both gas chromatography-mass spectrometry and direct injection. We propose that the pyrophosphate originated with the elimination of PPi from IPP during preparation of the TMS derivatives. Both of these measurements confirm the presence of IPP as the product of the enzymatic reaction.

For NMR analysis of the isopentenyl phosphate kinase reaction, 0.28 µg of purified enzyme was mixed with 250 mM Tris buffer (pH 7.5) containing 2.25 mM ATP, 2.25 mM MgCl$_2$, 2.25 mM IP, and 0.5 mM 2-mercaptoethanol in a final volume of 2 ml. The control was identical to the reaction mixture but contained no enzyme. The samples were incubated for 30 min at 55°C and then concentrated to 0.5 ml under a stream of nitrogen gas. ³¹P NMR spectra were obtained on a Varian Unity-400 NMR spectrometer at ambient temperature. The following ³¹P NMR assignments were made referenced to 85% H$_3$PO$_4$ for the proton-decoupled spectrum: ATP δ, −5.1 (1P, d, $J_{\gamma P \to \beta P}$ = 15 Hz, γP), −10.2 (1P, d, $J_{\alpha P \to \beta P}$ = 15 Hz, αP), and −19.2 (1P, m, βP); ADP δ, −5.54 (1P, d, $J_{\beta P \to P}$ = 19.9 Hz, βP) and −9.7 (1P, d, $J_{\alpha P \to \beta P}$ = 19.9 Hz, αP); IPP δ, −5.48 (1P, d, $J_{\beta P \to \alpha P}$ = 19.9 Hz, βP) and −9.3 (1P, d, $J_{\alpha P \to \beta P}$ = 19.9 Hz, αP); and IP δ, 4.38 (1P, s).

RESULTS AND DISCUSSION

Identification of an archaeal IP kinase. On the basis of chromosomal clustering analysis of archaeal mevalonate pathway genes (27, 32), we originally considered the protein product of the MJ0044 gene to be a phosphomevalonate kinase. MJ0044 is a member of a conserved orthogonal group of predicted archaeal kinases (COG1608). This would be yet another example of nonorthologous gene displacement as found in archaeal shikimate metabolism (12). The MJ0044 gene was cloned and overexpressed in *E. coli*. The resulting purified protein appeared as a single band at approximately 30 kDa, in good agreement with the predicted molecular mass of 29.5 kDa, and was purified to >98% homogeneity as judged by polyacrylamide gel electrophoresis with Coomassie blue staining. After repeated attempts we were unable to detect ATP-dependent kinase activity with the MJ0044 gene product when either mevalonate or mevalonate-5-phosphate was provided as a substrate. The close genomic association of this gene with genes for other mevalonate pathway enzymes led us to consider that an alternate pathway from mevalonate-5-phosphate to IPP may function in the archaea. Since having the second phosphate on mevalonate diphosphate would not appear to be required for the decarboxylation reaction (18), we then considered that mevalonate phosphate may in fact be the substrate for the decarboxylation reaction. This scenario would result in the formation of IP from mevalonate phosphate, which would then need to be phosphorylated to generate the final product, IPP (Fig. 2). We proposed that the MJ0044 gene product may be involved in this final transformation.

Incubation of the MJ0044 gene product with IP and ATP resulted in the formation of ADP, detected through the coupled pyruvate kinase-lactate dehydrogenase assay (1). The formation of ADP by the MJ0044 gene product was dependent on the presence of IP and was linear with respect to time and enzyme concentration. The formation of ADP was not observed in the presence of mevalonate or mevalonate phosphate. Incubation of ATP and IP with the enzyme (0.28 µg) in pH 7.5 Tris buffer for 30 min at 55°C resulted in the conversion of 22% of the IP to IPP and 22% of the ATP to ADP as measured by ³¹P NMR. This was confirmed by the loss in the intensities of IP resonance and α, β, and γ resonances for ATP and the corresponding increase in the resonances for IPP and ADP (Fig. 3). No inorganic pyrophosphate was detected in the incubation mixture by ³¹P NMR before or after incubation with the enzyme, and inorganic phosphate did not exceed 4% of the ATP concentration in either the control or sample reactions. This demonstrated that the MJ0044 gene product is not an ATP hydrolase.

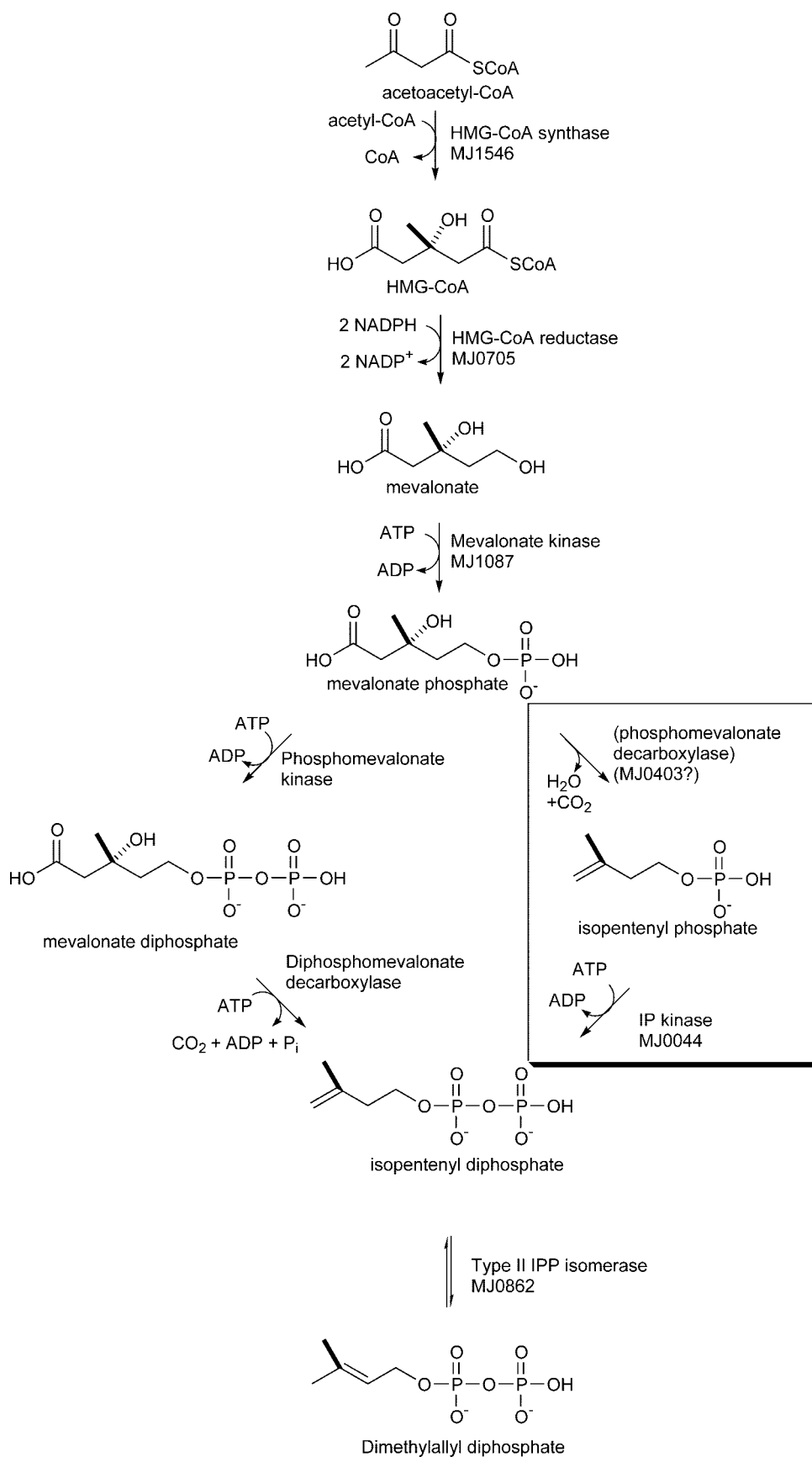In order to further confirm that the product of the MJ0044

FIG. 2. The mevalonate pathway. The proposed archaeal modifications of the mevalonate pathway, including the reaction catalyzed by IP kinase, are indicated in the box.
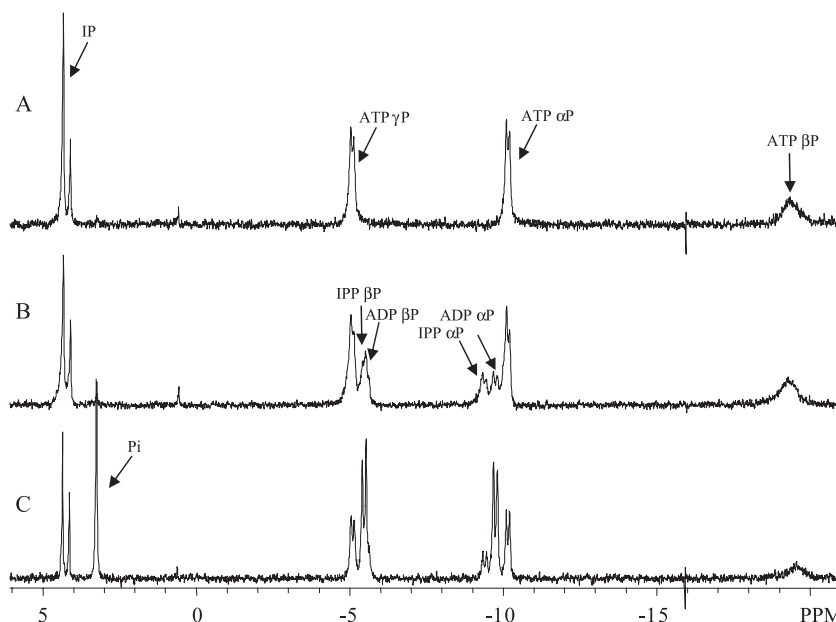
FIG. 3. ^{31}P NMR of IP kinase reaction products. The control reaction (A) and the IP kinase reaction (0.28 µg enzyme) (B) each contained 9 mM IP, 9 mM ATP, and 9 mM MgCl$_2$ and were incubated at 55°C for 30 min. After the spectrum shown in panel B was recorded, the sample was spiked with 9 mM ADP and 9 mM Pi and the spectrum shown in panel C was recorded. The intensity of the monophosphate ester at δ 4.2 (AMP?) did not change throughout the experiment.

reaction was the predicted IPP, the reaction mixture was subjected to MALDI mass spectral analysis. The MALDI mass spectrum of the reaction mixture showed the presence of an [M-H]$^-$ ion at *m/z* 245, the expected value for IPP. This ion was not present in the control reaction lacking the enzyme. The formation of IPP was further confirmed by the mass spectral detection of the diphosphate (TMS)$_4$ derivative likely formed by elimination of PPi from IPP during the attempted formation of the IPP (TMS)$_3$ derivative.

We found *M. jannaschii* IP kinase to have an apparent $K_{m,IP}$ of 256 µM, with a k_{cat} of 3.3×10^2 sec^{-1}. Although the $K_{m,IP}$ value is higher than expected, it falls in line with the values reported for mevalonate kinase from rats, humans, and *M. jannaschii*, which range from a $K_{m,R,S\text{-mevalonate}}$ of 68 to 288 µM (17). IP kinase was active over a broad pH range between 7 and 9, with an optimal pH around 9.0. IP kinase also retained 38% activity when incubated at 100°C for 10 min. Although we have identified several enzymes from *M. jannaschii* that exhibit a preference for GTP over ATP (16, 23), IP kinase showed a strict specificity for ATP.

This is the first report of an isopentenyl phosphate kinase associated with the mevalonate pathway. In eukaryotes that utilize the mevalonate pathway exclusively, an IP kinase is not required, as formation of the diphosphate occurs prior to the decarboxylation reaction. An isopentenyl phosphate kinase in *E. coli* has been reported previously (21); however, this enzyme was later found to be a 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase involved in the DXP pathway (24). Further analysis of a more purified enzyme preparation showed that the *E. coli* enzyme did not possess isopentenyl monophosphate kinase activity (30). There is no significant sequence similarity between *M. jannaschii* IP kinase and *E. coli* 4-diphosphocytidyl-2-C-methyl-*D*-erythritol kinase. Additionally, these two ki-

nases appear to be members of different protein families (10, 11). *M. jannaschii* IP kinase is predicted to be a member of pfam00696, the aspartokinase superfamily of kinases, and contains the conserved ATP binding residues common to this family of proteins (10, 11, 25). This is in contrast to other isoprene kinases involved in isoprene biosynthesis such as mevalonate kinase, phosphomevalonate kinase, and 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, which are members of the GHMP-kinase family of proteins (2, 21).

Archaeal modification of the mevalonate pathway. The presence of an IP kinase, in conjunction with the apparent absence of genes for phosphomevalonate kinase and diphosphomevalonate decarboxylase, suggests that an alternate pathway for the conversion of mevalonate-5-phosphate to IPP may be functioning in archaea. We propose that mevalonate phosphate may serve as the substrate for an unknown phosphomevalonate decarboxylase, resulting in the formation of IP (Fig. 2). The IP kinase that we report here would then represent the final step in the formation of IPP in archaea.

This proposed archaeal modification of the mevalonate pathway would still require a decarboxylase to convert mevalonate-5-phosphate to IP. We have identified an open reading frame, MJ0403, which may be involved in this transformation. MJ0403 homologs are colocalized with other genes for mevalonate pathway enzymes on multiple archaeal chromosomes (Fig. 1). In several archaea, including *Methanobacterium thermoautotrophicum*, *Methanosarcina mazei*, and *Aeropyrum pernix*, this gene is found in close proximity to the IP kinase and other mevalonate pathway genes (Fig. 1). MJ0403 is annotated as a dioxygenase and has the conserved dioxygenase iron binding site; however, overall sequence similarity is quite low.

Evolutionary implications. Despite the apparently clear distinction between organisms that operate the mevalonate versus

those that operate the DXP pathway, there is much evidence for lateral gene transfer of many isoprene biosynthetic genes. In the archaea, evidence for lateral gene transfer is found in the phylogenetic relationships between the types of HMG-CoA reductase and of IPP isomerase as well as diphosphomevalonate decarboxylase (if present) (6, 7). Lateral gene transfer has been suggested to occur for IPP isomerase, and examples exist in both the archaea and bacteria where type I, type II, or both or neither isomerase is present (7, 19). Many species of the *Halobacteria* have homologs of type I and type II IPP isomerases (3, 7, 26). Similarly, species of both *Sulfolobales* and *Halobacteriales* have homologs of diphosphomevalonate decarboxylase; however, the *Sulfolobales* enzyme resembles the eukaryotic type while the *Halobacteriales* enzyme more closely resembles the bacterial enzyme, suggesting that they were obtained from different sources (7). Additional evidence for lateral gene transfer was found in the types of HMG-CoA reductases by Boucher et al. (6). While eukaryotes and most archaea have a class 1 HMG-CoA reductase, some archaea, such as the *Archaeoglobales* and the *Thermoplasma*, have a class II bacterial HMG-CoA reductase (6).

Taken together, it appears that lateral gene transfer of mevalonate pathway genes is not uncommon in the archaea. It is of note that, with the exception of species of *Sulfolobales*, phosphomevalonate kinase homologs have not been identified in the archaea, particularly those presenting evidence of lateral gene transfer in other mevalonate pathway genes. Also note that species of both *Sulfolobales* and *Halobacteriales*, as well as other archaea examined, possess an IP kinase homolog. The presence of this enzyme would suggest that the production of IP as a precursor to IPP occurs in these organisms. The significance of this may be better addressed after the complete archaeal pathway for IPP biosynthesis has been elucidated.

Conclusions. The absence of enzyme homologs for two central steps of the mevalonate pathway in the archaea is conspicuous, especially in the context of the apparent abundance of gene transfer events. This, coupled with the presence of the IP kinase described in this paper, suggests that these organisms are using an alternate route to synthesize IPP. The possibility that the established mevalonate pathway is functioning in the archaea can not be ruled out at this point, however. The archaeal homologs of phosphomevalonate kinase and diphosphomevalonate decarboxylase simply may not possess enough sequence similarity with known enzymes to be readily identified. Additional study of archaeal isoprene biosynthesis is currently under way in our laboratory in an attempt to unravel the answers to some of these questions.

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