Characterization of phosphomevalonate kinase: chromosomal localization, regulation, and subcellular targeting

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Abstract Phosphomevalonate kinase catalyzes the conversion of mevalonate-5-phosphate to mevalonate-5-diphosphate and was originally believed to be a cytosolic enzyme. In this study we have localized the phosphomevalonate kinase gene to chromosome 1p13–1q22–23 and present a genomic map indicating that the gene spans more than 8.4 kb in the human genome. Furthermore, we show that message levels and enzyme activity of rat liver phosphomevalonate kinase are regulated in response to dietary sterol levels and that this regulation is coordinate with 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme of cholesterol biosynthesis. In addition, we demonstrate that phosphomevalonate kinase is a peroxisomal protein which requires the C-terminal peroxisomal targeting signal, Ser-Arg-Leu, for localization to the organelle.—Olivier, L. M., K. L. Chambliss, K. M. Gibson, and S. K. Krisans. **Characterization of phosphomevalonate kinase: chromosomal localization, regulation, and subcellular targeting.** ***J. Lipid Res.*** **1999.** 40: **672–679.**

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Peroxisomal matrix proteins are targeted to the organelle by specific targeting sequences. Two such peptides for peroxisomal protein targeting have been characterized. The first identified peroxisomal targeting signal (PTS-1), is a tripeptide with the consensus sequence of (S/A/C) (K/H/R) (L/M), found at the extreme carboxyl terminal of peroxisomal proteins (1, 2). The second targeting signal, PTS-2, is a nine amino acid sequence (R/K)(L/V/I)(X5)(H/Q)(L/A) found in the amino-terminal region of a smaller subset of peroxisomal matrix proteins (3). Receptors for each of these sequences have been cloned and protein import studies indicate these receptors are essential for peroxisomal protein import. Moreover, mutations in either receptor are responsible for several of the fatal human peroxisomal deficiency disorders (4, 5).

In contrast to their previously believed cytosolic location, several enzymes in the cholesterol biosynthetic pathway have recently been discovered to reside in peroxisomes. Specifically, enzymatic activities for acetoacetyl CoA thiolase (6), HMG-CoA synthase (7), HMG-CoA reductase (8, 9), mevalonate kinase (MvK) (10), and farnesyl diphosphate synthase (FPPS) (11) have been localized to peroxisomes. Both MvK and FPP synthase have been shown by immunofluorescence and immunoelectron microscopy to be predominantly peroxisomal (10, 11). Furthermore, data obtained from enzyme assays on peroxisome-deficient cells and digitonin-permeabilized cells offer indirect evidence suggesting the possible peroxisomal location of phosphomevalonate kinase (PMvK) and mevalonate pyrophosphate decarboxylase (MPD) (11, 12). In addition, the cloning of human MvK (13), PMvK (14), MPD (15) and isopentenyl diphosphate dimethylallyl diphosphate isomerase (IPPI) (16) has led to the identification of putative peroxisomal targeting signals contained in their amino acid sequences. Interestingly, the analysis of the amino acid sequence of IPPI indicated the presence of both a putative PTS-1 and PTS-2 motif. However, we have recently shown that the protein is targeted to the peroxisomes through the PTS-1 motif (17). Therefore, collectively these data suggest that peroxisomes may contain all the enzymatic reactions required for the biosynthesis of farnesyl diphosphate from acetyl-CoA.

PMvK catalyzes the conversion of mevalonate 5-phosphate into mevalonate 5-diphosphate as the fifth reaction of the

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; FPPS, farnesyl diphosphate synthase; MvK, mevalonate kinase; PMvK, phosphomevalonate kinase; MPD, mevalonate pyrophosphate decarboxylase; IPPI, isopentenyl diphosphate dimethylallyl diphosphate isomerase; LDL, low density lipoprotein; PCR, polymerase chain reaction; UTR, untranslated region; STS, sequence tagged sites; SRE, sterol regulatory element; GAPDH, glyceraldehyde-3-dehydrogenase; GFP, green fluorescent protein.

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cholesterol biosynthetic pathway. Recently the cDNA for human PMvK has been cloned and sequenced (14). PMvK is an 827 bp cDNA expressed in a variety of tissues with high expression in heart, skeletal muscle, liver, kidney, and pancreatic tissues. The human PMvK sequence contains a putative PTS-1, SRL, at the extreme carboxy terminal. Analysis of PMvK message from lymphoblasts grown in lipoprotein-deficient media or media containing lovastatin, an inhibitor of HMG-CoA reductase, indicated that PMvK expression may be responsive to lipid availability (14).

In this study we have identified the chromosomal location of PMvK and present a gene map for the vast majority of the PMvK genomic sequence. We provide evidence in vivo that PMvK mRNA levels and the corresponding enzymatic activity are regulated in response to cellular sterol levels. In addition, we demonstrate that PMvK is a peroxisomal protein which utilizes a PTS-1 for its peroxisomal localization.

MATERIALS AND METHODS

Materials

Restriction enzymes were purchased from New England Biolabs and all biochemicals were purchased from Sigma. AGI-X8 200–400 mesh formate resin was purchased from Bio-Rad. (RS)-[5-^{3}H]mevalonic acid, (R)-[2-^{14}C]mevalonic acid 5-phosphate, [1-^{14}C]isopentenyl diphosphate were purchased from American Radiolabeled Chemicals Inc. dl-3-[Glutaryl-3-^{14}C]hydroxy-3-methylglutaryl coenzyme A was purchased from DuPont NEN.

Chromosomal location

Chromosomal localization of PMvK was determined by polymerase chain reaction (PCR) amplification of genomic DNA from a human/hamster monochromosomal somatic cell hybrid line (BIOS) using PMvK specific primers. The primers used were 5′-CCTCAGCTTCCCACTTTAG-3′ upstream, which resides within intron 2 and 5′-TCCGAAAGGCCTCCTTGTAG-3′ downstream, located within exon 3.

Isolation of genomic PMvK

The genomic PMvK was isolated by screening a human λ DASH II library (Stratagene). Full-length PMvK cDNA labeled with ^{32}P was used to screen plaque lifts using C/P lift membranes (Bio-Rad). Approximately 2×10^{6} plaques were screened to yield the PMvK clone. Phage DNA was isolated using the Qiagen Lambdaprep Kit.

Animal studies

Male Sprague-Dawley rats were fed three different diets, three rats per diet. The first diet consisted of standard rat chow. The second diet consisted of standard rat chow supplemented with 5.0% cholestyramine for 2 days followed with 5.0% cholestyramine and 0.1% mevinolin for 3 days. The third diet consisted of the standard rat chow supplemented with 2% cholesterol for 15 days. The rats were maintained in a 12-h light/dark cycle and were killed 4–6 h into the light cycle. Isolated livers were homogenized in buffer containing 0.25 mm sucrose, 5 mm Tris-HCl, and 1 mm EDTA, pH 7.5. In addition, a protease inhibitor cocktail was added which consisted of 1.28 μg/ml aprotinin, 10 μg/ml cyclohexamide, 125 ng/ml pepstatin A, 250 μg/ml antipain, 125 ng/ml chymostatin, 250 ng/ml leupeptin, 100 μm PMSF, 20 μm DTT, 2 mm methionine (in sucrose), 15 μg/ml calpain I, and 15 μg/ml calpain II.

Northern blot

Poly(A)$^{+}$ RNA was isolated from 2.0 g of liver from each animal using standard RNA isolation techniques (18). These RNA samples were transferred from an agarose gel onto a Zeta probe GT membrane (Bio-Rad) using a Posiblot Pressure Blotter (Stratagene). A 601 bp Pst I/Xba I fragment of human PMvK was labeled with ^{32}P dCTP (NEN DuPont) by Nick translation as per manufacturers methods (Boehringer Mannheim). The Northern blot was incubated with the PMvK probe in hybridization solution (50% formamide, 6% SDS, 100 mm $(Na)_3PO_4$ and 200 mm NaCl). After overnight incubation at 45°C, the blot was washed three times at 50°C in 0.5× SSC/0.1% SDS. The blot was exposed on a PhosphorImager screen overnight (Molecular Dynamics) and quantitated the next day using Image Quant (MolecularDynamics).

Phosphomevalonate kinase assay

Reactions were carried out in a buffer containing 100 mm phosphate buffer, 4 mm $MgCl_2$, 1 mm dithiothreitol, 1 mm EDTA, 2% Tx-100 and ATP, pH 7.4. [^{14}C]mevalonic acid 5-phosphate was added at 2.0×10^{4} dpm/nmol. Five μg of homogenate liver samples was added and the samples were incubated for 15 min at 37°C. Phosphorylated products were separated on AGI-X8 200–400 mesh formate resin columns and sequentially washed with 4 N formic acid, to remove remaining substrate, followed by 0.8 m ammonium formate, to elute the phosphorylated products (11).

HMG-CoA reductase assay

The reaction was run as described (19) with the following minor modifications. Briefly, 100-μg samples of homogenized liver were incubated in phosphate buffer containing 200 mm NaCl, 30 mm EDTA, 10 mm DTT, 2 mm NADPH, 20,000 dpm [^{3}H]mevalonic acid and 200 μm [^{14}C]HMG-CoA. The reactions were run at 37°C for 40 min. Products were separated on AGI-X8 200–400 mesh formate resin columns.

Isopentenyl diphosphate isomerase assay

Homogenized liver samples were assayed in the following reaction mix: 10 mm HEPES, 0.5% Tx-100, 10 mm 2-mercaptoethanol, 1 mm $MgCl_2$, 0.01% BSA, and 22 dpm/pmol [^{14}C]IPP. Reactions were incubated for 10 min at 37°C and stopped with 2 volumes 25% HCL in methanol. Products were separated by hexane extraction (20).

Sequence analysis

The sequence for mouse PMvK was acquired by a GenBank EST search and can be found under accession numbers AA060815 and AA270706.

Cell culture

Chinese hamster ovary (CHO) cells were maintained in DMEM/F12 containing 5% FBS (Life Technologies), 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate at 37°C in a 5% CO_2 incubator. The peroxisomal-deficient transformed fibroblasts were a generous gift from Dr. Suresh Subramani. Both of these fibroblast cell lines were maintained in DMEM/F12 containing 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate at 37°C in a 5% CO_2 incubator.

Subcellular localization of PMvK

The expression vector pEGFP-C3 was purchased from Clontech. A 600 bp fragment of human PMvK was directionally subcloned into Pst I/Xba I sites of pEGFP-C3 to generate pEGFPC3-

PMvK. The ligated product was transformed into Top 10F′ *E. coli* (Invitrogen), grown, and the DNA was purified using a Qiagen Miniprep kit. In order to generate pEGFPC3-PMvKΔPTS-1, human PMvK was used as a PCR template. The PMvK specific PCR primers were gPM5P1 5′-GATGTCCCCGACTATGCCCCGGGA-3′ and 3′p1 5′-CTGATAGAATTTATCCGCTAGGGTACCGA-3′ (Gibco BRL). The resulting PCR product was then digested with Pst I and Kpn I, ligated into pEGFP-C3, transformed and prepped as described for pEGFPC3PMvK. Content of constructs was analyzed by restriction digest.

Expression vectors were transfected into cultured fibroblasts using Lipofectamine (Gibco BRL). The transfection media consisted of 1 ml DMEM, 1 μg vector DNA, and 6 μl Lipofectamine reagent which was incubated on the cells for 2 h at 37°C. After this 2-h incubation period, 1 ml of DMEM/F12 containing 10% FBS, 200 units penicillin, and 200 μg streptomycin sulfate was added to the culture media and incubated overnight.

After transfection, cells were fixed onto coverslips with 3.0% paraformaldehyde in PBS for 15 min. Cells were then permeabilized with 1% Triton X-100 for 5 min. Catalase was detected using rabbit anti-catalase antibody at a 1:200 dilution in 1% BSA/PBS solution incubated on the cells for 1 h. After washing the coverslips with PBST, a Texas red goat anti-rabbit secondary antibody at 1:200 dilution was incubated for 1 h. Finally, after washing with PBST, the coverslips were mounted with slowfade Antifade Kit (Molecular Probes) and visualized with a Nikon Fluorescent microscope.

RESULTS AND DISCUSSION

Chromosomal location and genomic map for human PMvK

Chromosomal localization of PMvK was determined by PCR amplification of PMvK in a human/hamster monochromosomal cell line using PMvK specific primers. The 430 bp amplicon was seen in control human genomic DNA and in the somatic cell hybrid carrying human chromosome 1 (**Fig. 1**). The amplicon was not seen in control hamster genomic DNA nor in controls lacking DNA template. A search of sequence tagged sites (STS) by electronic PCR at the National Center for Biotechnology Information (NCBI) confirmed and refined the PMvK position on human chromosome 1. A 399 bp fragment at the 3′ extremity of the PMvK cDNA was 100% homologous to STS WI-14846. The STS has been mapped to 164–170 centiMorgans on chromosome 1, located between markers D1S305 and D1S2635. This localizes the PMvK gene at human chromosome 1p13–1q22–23.

A human PMvK genomic clone containing an 8,427 bp insert was isolated from a human λ DASH II library after screening 1×10^6 lambda phage. The insert contained four PMvKase exons encoding 162 of the 192 amino acid residues of the protein and the 3′ untranslated region (UTR) including 620 bp past a consensus polyadenylation signal (**Fig. 2**). This sequence has been deposited in the GenBank with the accession number AF026069. The 5′UTR and first 30 amino acid residues were not present in the genomic clone. Considerable effort has been expended in order to complete the genomic sequence for PMvK. An additional 10^6 phage were screened and a "Long PCR" technique was also undertaken using the lambda library as template in order to obtain DNA corresponding to the missing 30 amino acids. However, neither of these techniques yielded any additional PMvK clones. Therefore, it is likely that this genomic library does not contain inserts that fully represent the genomic sequence of PMvK.

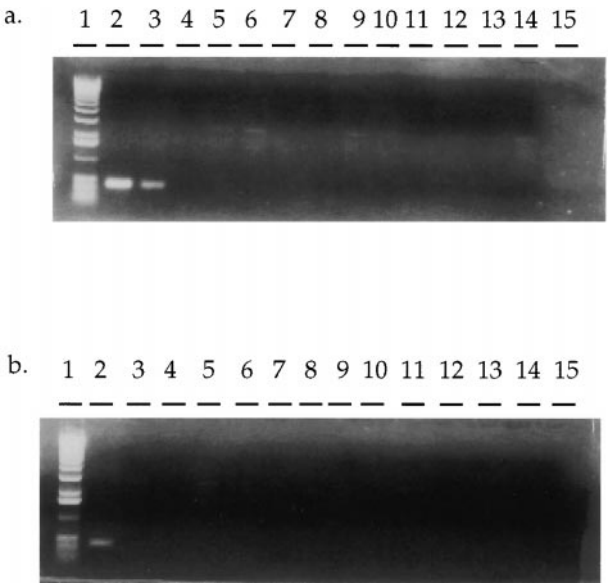

Fig. 1. PMvK is located on human chromosome 1. (a) The first lane is a 1 kb molecular weight ladder. The second lane is control human genomic DNA showing the 430 bp PMvK PCR product. Lanes 3–15 correspond to PCR products from monochromosomal hybrids carrying human chromosome 1 (lane 3), 2 (lane 4), 3 (lane 5), 4 (lane 6), 5 (lane 7), 6 (lane 8), 7 (lane 9), 8 (lane 10), 9 (lane 11), 10 (lane 12), 11 (lane 13), and 12 (lane 14). Lane 15 is blank. (b) The first lane is a 1 kb molecular weight ladder and the second lane is control human genomic DNA showing the 430 bp PMvK PCR product. The bottom set of wells begins with chromosome 13 (lane 3), 14 (lane 4), 15 (lane 5), 16 (lane 6), 17 (lane 7), 18 (lane 8), 19 (lane 9), 20 (lane 10), 21 (lane 11), 22 (lane 12), 23 (lane 13), X (lane 14), and Y (lane 15).

Regulation of PMvK gene expression

Cellular cholesterol levels are tightly regulated allowing for a constant level to be maintained. The expression of many enzymes in the cholesterol biosynthetic pathway is regulated in response to dietary cholesterol levels via a sterol regulatory element (SRE) found within the promoter region. The initial studies describing SREs characterized the LDL receptor promoter (21) and mutational analysis defined the 10 bp SRE sequence as 5′-ATCACCCCAC-3′ (22). Three transcription factors, encoded by two genes, sterol regulatory element binding proteins 1a, 1c, and 2, recognize and bind the SREs (22, 23). Genes identified as being responsive to cellular sterol levels include HMG-CoA synthase (24), HMG-CoA reductase (25), MvK (26), squalene synthase (27), FPPS (28), fatty acid synthase (29), acetyl-CoA carboxylase (30), and the LDL receptor (31).

The initial studies of PMvK expression suggested that transcription of PMvK was responsive, in lymphoblasts, to

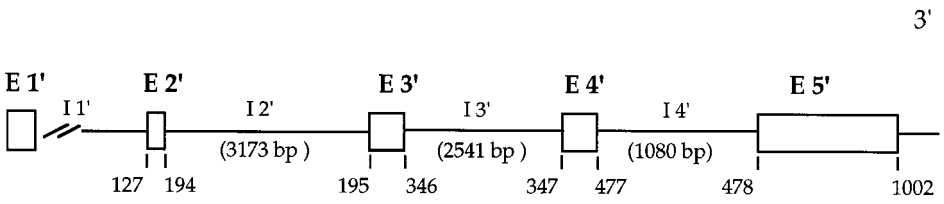

Fig. 2. PMvK gene structure. The genomic structure of PMvK is shown with boxes representing current numbering of exons (E′ 1–5) and the connecting lines as introns (I′ 1–4). Slashes across the gene indicate the small area of the clone which is missing. Base pair numbering of the exons corresponds to the cDNA sequence with the intronic sequence numbering in parentheses.

lipid availability (14). However, a rigorous analysis of PMvK expression was required to definitively determine its regulation within the cholesterol biosynthetic pathway. Therefore, we were interested in determining the effect of lipid-modifying diets on PMvK expression in vivo. Northern blot analysis of PMvK showed the expression level of the approximately 1 kb PMvK transcript, after cholesterol feeding, was half of controls. Furthermore, expression of PMvK increased by 10-fold over controls with the cholestyramine and mevinolin (C+M) diet (**Fig. 3A** and **B**). Expression of glyceraldehyde-3-dehydrogenase (GAPDH) is unaffected by cholesterol levels and was used as a control for sample loading. From these results it is evident that the expression of PMvK in vivo is regulated in response to cholesterol levels consistent with the regulation determined for other cholesterol biosynthetic enzymes.

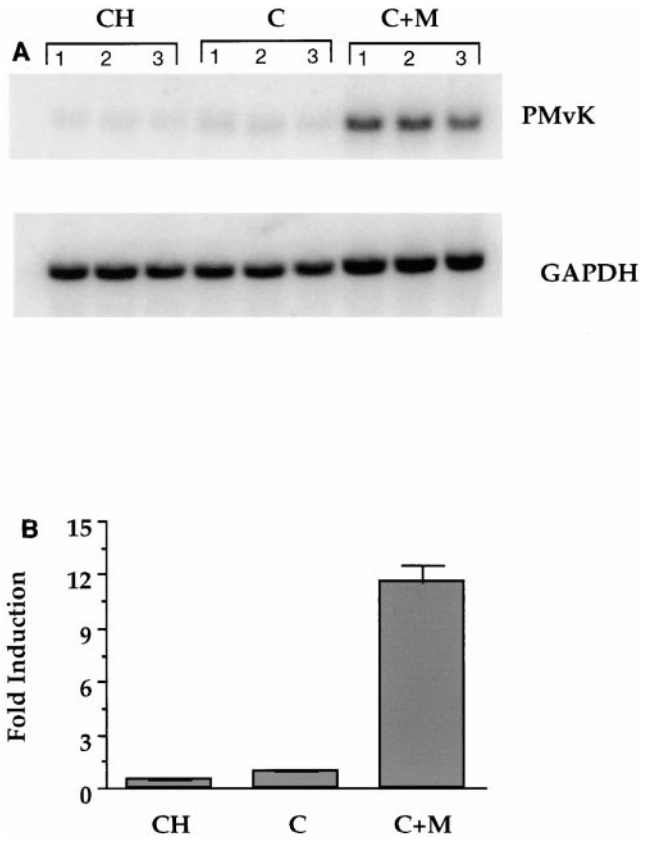

Fig. 3. Regulation of rat PMvK mRNA by dietary sterol levels. (A) Northern blot analysis of 10 μg rat liver Poly (A)$^+$ mRNA probed with human PMvK cDNA. Rat diets were as follows: CH, 2% cholesterol diet, C, control diet, and C+M, 5% cholestyramine +0.1% mevinolin. The blot was stripped and reprobed with glyceraldehyde 3-dehydrogenase. (B) Imagequant analysis of the PMvK expression levels in response to the diets, values indicate fold induction relative to control mRNA expression (set as 1) and corrected for GAPDH expression.

Enzymatic activities

In order to further examine the regulation of PMvK by cellular sterol levels the enzymatic activity level of PMvK was determined. PMvK activity measurements from homogenized liver samples, fed the same diet as for the Northern analysis, were consistent with the data found at the mRNA level. Cholesterol-enriched diets slightly decreased PMvK activity whereas those fed the C+M diet increased their expression 4-fold over controls (**Table 1**). In order to determine whether the regulation of PMvK enzyme activity relates to other enzymes in the cholesterol biosynthetic pathway, these samples were also assayed for HMG-CoA reductase and IPPI. HMG-CoA reductase is the rate-limiting step in the cholesterol biosynthetic pathway and its regulation has been extensively studied (32–34). On the other hand, while the regulation of IPPI transcription has been studied by Northern analysis, there is no corresponding enzymatic data available. As expected, HMG-CoA reductase activity was significantly reduced compared to control rats fed the cholesterol diet and was induced 26-fold over controls with the C+M diet. IPPI activity was also reduced with cholesterol feeding and induced 13.1-fold over control rats fed the C+M diet. Therefore, all three enzymes, PMvK, HMG-CoA reduc-

TABLE 1. Dietary regulation of enzymatic activity

	PMvK	HMG-CoA Reductase	IPPI
	nmol/min/mg	*pmol/min/mg*	*pmol/min/mg*
Control	14.9 (±2.5)	23.0 (±1.6)	326.4 (±29.1)
C+M	73.5 (±7.8)	604.0 (±50.7)	4278.2 (±405.4)
Cholesterol	13.1 (±1.4)	4.2 (±0.3)	80.6 (±3.4)

Nine rats were fed cholesterol-modifying diets. Three rats were fed each of the following diets: a standard diet (control), a diet supplemented with 2% cholesterol (CH), or a diet supplemented with cholestyramine and mevinolin (C+M). The average specific activity is indicated for each assay with the standard error in parentheses.

tase, and IPPI are coordinately regulated in response to dietary cholesterol levels.

Subcellular localization of PMvK

In the initial sequence analysis of human PMvK, a putative PTS-1 was identified. Furthermore, upon comparison of the human amino acid sequence to the mouse amino acid sequence, it is evident that this targeting signal is conserved between the species as both contain the consensus PTS-1 sequences (**Fig. 4**). Interestingly, these species are 83% identical over the 192 amino acid sequence and 86% identical in the nucleotide sequence. Due to the presence of the putative peroxisomal targeting signal in both human and mouse amino acid sequence, the functionality of this motif was investigated.

Green fluorescent protein (GFP) is a cytosolic protein isolated from the jellyfish *Aequorea victoria.* Several studies have demonstrated that attachment of a subcellular targeting signal to GFP results in the fluorescent fusion protein residing in the corresponding subcellular compartment (35, 36). In order to study the subcellular localization of PMvK, two GFP fusion proteins were generated (**Fig. 5a**). One fusion protein was produced by attaching a 600 bp Pst I-Xba I fragment of human PMvK cDNA, containing the putative PTS-1, to the carboxyl terminus of the green fluorescent protein (pEGFP-PMvK). The other GFP fusion protein construct was generated similarly; however, in this construct the last three amino acids of PMvK, corresponding to the putative PTS-1, were removed (pEGFP-PMvKΔPTS).

Transient transfection of pEGFP-PMvK into CHO cells produced a punctate labeling distribution, indicative of peroxisomal localization (Fig. 5b). Furthermore, this punctate pattern was superimposable onto the pattern for the peroxisomal marker enzyme, catalase within the same cell (Fig. 5c), identifying the peroxisomal localization of this fusion protein. In order to determine whether the identified putative PTS-1 is required for PMvK localization to the peroxisome the second construct, pEGFP-PMvKΔPTS-1, was transfected into CHO cells. Transfection of this construct resulted in a cytosolic distribution of fluorescence (Fig. 5d), while the catalase labeling remained clearly peroxisomal (Fig. 5e). Therefore, this inability to target pEGFP-PMvKΔPTS-1 to peroxisomes was clearly not due to a cellular abnormality as catalase localization was peroxisomal. Furthermore, considering the only difference between these two fusion proteins is the putative PTS-1 SRL, it is evident that the carboxy-terminal tripeptide is necessary for peroxisomal targeting.

For further verification of the peroxisomal localization and the import system utilized for PMvK, we transfected pEGFP-PMvK in PTS-1-deficient fibroblasts. Peroxisomal import-deficient fibroblasts have become a useful tool in the study of peroxisomal protein localization. These fibroblasts, isolated from an individual with a peroxisomal deficiency disorder, have a mutation in the PTS-1 receptor

Fig. 4. Human and mouse PMvK sequence alignment. Mouse PMvK amino acid sequence is shown with the human sequence variation indicated below. All other amino acids are identical. The C-terminal sequence for both species of PMvK contains a peroxisomal targeting signal-1 (boxed).

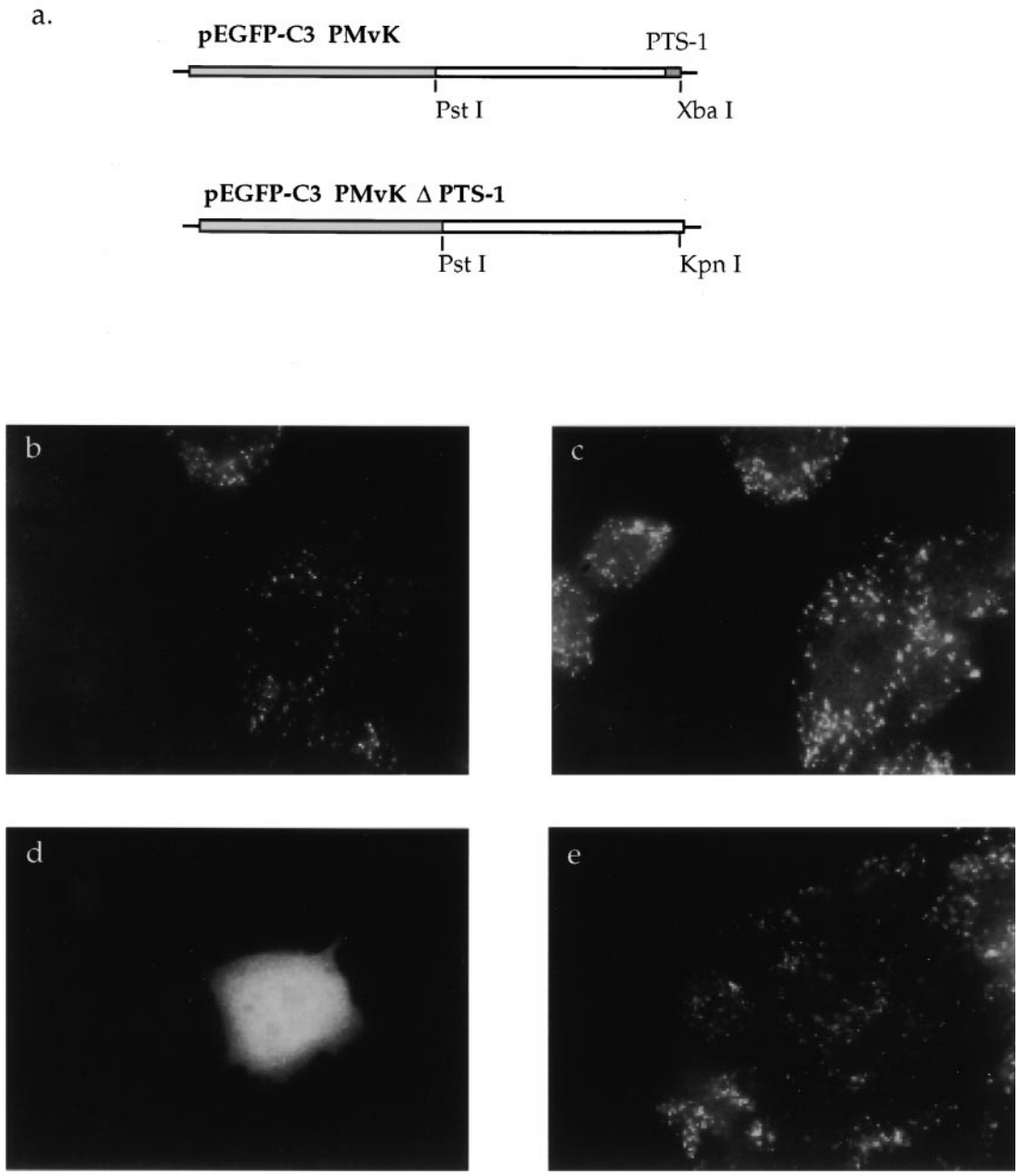

Fig. 5. PMvK localizes to peroxisomes and the PTS-1 is required for PMvK peroxisomal import. (a) PMvK fusion vector construction. The PMvK PstI/XbaI fragment was subcloned in frame with pEGFP-C3, yielding the vector pEGFP PMvK. PCR was performed using specific primers truncating PMvK immediately before the PTS-1 sequence. This fragment was then cut with Pst I and Kpn I and subcloned in frame with EGFP, yielding the vector pEGFP PMvKΔPTS-1. Subcellular localization of the fusion proteins in CHO cells was determined by colocalization of the GFP with catalase. (b) Transiently transfected pEGFP PMvK resulted in a punctate distribution of fluorescence which was superimposable on (c) the immunofluorescent pattern of the peroxisomal marker, catalase. pEGFP PMvKΔPTS-1 was also transiently transfected and resulted in cytosolic labeling (d) whereas the corresponding catalase distribution remained punctate (e).

that results in the inability to import PTS-1-containing proteins (4). In addition, the construct was also transfected into PTS-2-deficient fibroblasts in which PTS-2-containing proteins are not imported due to a mutation in the PTS-2 receptor (5). In both cases the inability to localize proteins to the peroxisomes results in their cytosolic localization. Transfection of pEGFP-PMvK in the PTS-1-deficient fibroblasts resulted in cytosolic labeling (**Fig. 6a**). This finding is consistent with the conclusion that PMvK is imported into peroxisomes in a PTS-1-dependent manner. Furthermore, transfection of the same construct into the PTS-2-deficient fibroblasts exhibited a punctate pattern of fluorescence indicative of peroxisomal localization (Fig. 6b). Therefore, these results demonstrate that PMvK is a peroxisomal protein that utilizes the carboxy terminal PTS-1 for its peroxisomal localization.

Cholesterol biosynthesis occurs through more than 30 discrete reactions located in the cytosol, endoplasmic reticulum, and peroxisomes (37). As mentioned previously, studies have shown that enzymatic activities for acetoacetyl Co-A

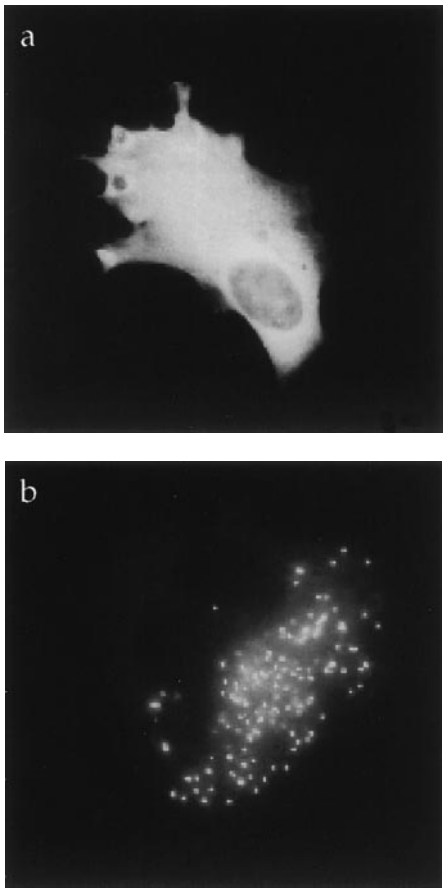

Fig. 6. PMvK utilizes the PTS-1 receptor for peroxisomal import. (a) A human fibroblast cell line deficient in PTS-1 protein was transiently transfected with pEGFP PMvK. Visualization of transfected cells shows a cytosolic distribution of the fusion protein. (b) The same construct was transiently transfected into a human fibroblast cell line deficient in PTS-2 protein import. Visualization of these cells indicates a punctate cellular distribution consistent with peroxisomal distribution.

thiolase (6), HMG-CoA synthase (7), HMG-CoA reductase (8, 9, 19), MvK (10), and FPPS (11) are localized to peroxisomes. In addition, data obtained from enzyme assays on peroxisome-deficient cells and digitonin-permeabilized cells offer indirect evidence suggesting the possible peroxisomal location of PMvK and MPD (11, 12). This is the first study to directly demonstrate that PMvK is a peroxisomal protein. Furthermore, mechanisms for peroxisomal targeting have only been elucidated for IPPI (17) and, now, in this study, for PMvK. Of the other three enzymes required for the conversion of mevalonate to farnesyl diphosphate, two of these, MvK (13) and MPD (15) may contain putative PTS-2s. Therefore, subsequent analyses of these enzymes will be required to determine which targeting signals are used.

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