Malonyl-CoA decarboxylase (MCD) catalyzes the proton-consuming conversion of malonyl-CoA to acetyl-CoA and CO₂. Although defects in MCD activity are associated with malonyl-CoA decarboxylase deficiency, a lethal disorder characterized by cardiomyopathy and developmental delay, the metabolic role of this enzyme in mammals is unknown. A computer-based search for novel peroxisomal proteins led to the identification of a candidate gene for human MCD, which encodes a protein with a canonical type-1 peroxisomal targeting signal of serine-lysine-leucine. We observed that recombinant MCD protein has high intrinsic malonyl-CoA decarboxylase activity and that a malonyl-CoA decarboxylase-deficient patient has a severe mutation in the MCD gene (c.947–948delTT), confirming that this gene encodes human MCD. Subcellular fractionation experiments revealed that MCD resides in both the cytoplasm and peroxisomes. Cytoplasmic MCD is positioned to play a role in the regulation of cytoplasmic malonyl-CoA abundance and, thus, of mitochondrial fatty acid uptake and oxidation. This hypothesis is supported by the fact that malonyl-CoA decarboxylase-deficient patients display a number of phenotypes that are reminiscent of mitochondrial fatty acid oxidation disorders. Additional support for this hypothesis comes from our observation that MCD mRNA is most abundant in cardiac and skeletal muscle, tissues in which cytoplasmic malonyl-CoA is a potent inhibitor of mitochondrial fatty acid oxidation and which derive significant amounts of energy from fatty acid oxidation. As for the role of peroxisomal MCD, we propose that this enzyme may be involved in degrading intraperoxisomal malonyl-CoA, which is generated by the peroxisomal β-oxidation of odd chain-length dicarboxylic fatty acids.

Malonyl-CoA decarboxylase activity (EC 4.1.1.9, Scheme 1) has been described in a wide array of organisms, including prokaryotes, birds, and mammals (1–3). However, the physiological role of this enzyme is somewhat unclear. The only eu-
Human Malonyl-CoA Decarboxylase

dria, DFAs are oxidized only in peroxisomes (9). DFA oxidation is poorly understood, and it is not clear whether peroxisomes degrade DFAs completely to malonyl-CoA (for odd chain-length DFAs) and oxaoyl-CoA (for even chain-length DFAs). However, if the β-oxidation of DFAs in peroxisomes is complete, a peroxisomal form of MCD could function to eliminate this metabolic end-product of odd chain-length DFA oxidation.

A key role for malonyl-CoA decarboxylase in mammalian metabolism is suggested by the severe phenotypes of patients who lack this enzyme activity. Malonyl-CoA decarboxylase deficiency, also known as malonic aciduria, is a genetic disorder characterized by developmental delay, cardiomyopathy, mental retardation, and in its more severe forms, neonatal death (10–14). These patients have several phenotypes that are reminiscent of mitochondrial fatty acid oxidation deficiencies, including diet-induced and infection-induced vomiting, seizures, hypoglycemia, and organic aciduria, as well as cardiomyopathy. Here we report the identification of a novel human gene encoding malonyl-CoA decarboxylase. Its role in malonic acid-mal protein, we searched for human homologues of goose MCD. The largest band that was generated was cloned between the SacI and NotI sites of pMPB, a variant of pMALe2 (New England Biolabs) that contains unique SacI and NotI sites for insertion of fragments downstream of the maltose-binding protein (MBP) ORF (16). The resulting plasmid, pMPB-MCD, was used for immunization of rabbits and generation of affinity purified anti-MCD antibodies. Another set of primers (5′-CCAGTCGACGAGCGCGGCCATGGG-3′ and 5′-CAAGTTTGACGACGGCCGCTCAAAGCTTCGAGTTC-3′) was used to amplify the entire MCD ORF in the correct reading frame for fusion to MBP in pMPB. The template for this reaction was a human heart cDNA library. A product of the expected length was cloned, cleaved with SacI and NotI, and cloned between the SacI and NotI sites of pMPB. The insert in this plasmid (pMPB-MCD) was sequenced in its entirety, confirming the full-length sequence assembled from the overlapping MCD cDNAs described in the cDNA cloning section above. This plasmid was used for generating full-length recombinant MCD that was used for enzyme assays. To create pcDNAs-MCD, the EcoRI-NotI fragment containing the MCD cDNA was excised from pMPB-MCD and inserted between the EcoRI and NotI sites of pcDNAs (Invitrogen, San Diego).

**Preparation of Lysates, In Vitro Translation, Subcellular Fractionation, and Immunoblotting**—Whole cell protein extracts for use in immunoblotting were prepared from cultured human HepG2 cells, a hepatoma cell line, and 575T6 cells, a human skin fibroblast cell line. A nearly confluent flask (150 cm²) of cells was resuspended in SDS-PAGE sample buffer. Coupled in vitro transcription and translation of human MCD was performed with TNT coupled to the MCD reaction to malate dehydrogenase and citrate synthase by making use of the product of the MCD gene from a Caenorhabditis elegans form of MCD (GenBank accession number Z46242). None of these are as long as our assembled “full-length” MCD cDNA.

**Experimental Procedures**—We searched the ExPASy TrEMBL/EMBL databases for all proteins terminating in possible forms of the PTS1. This data base scan identified goose malonyl-CoA decarboxylase as a candidate peroxisomal protein because it ends in the canonical PTS1 of serine-lysine-leucine-COOH (15). To determine whether the human gene also encoded a protein with a PTS1, and thus might encode a peroxisomal protein, we searched for human homologues of goose MCD. The goose MCD protein sequence was used as query in a BLAST search of the human data base of expressed sequence tags (ESTs) for any genes also encoding a protein with a PTS1, and thus might encode a peroxisomal protein because it ends in the canonical PTS1 of serine-lysine-leucine-COOH. We searched the ExPASy TrEMBL/EMBL databases for all proteins ending in possible forms of the PTS1.

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extracted from cultured fibroblast monolayers using PureScript reagents and protocols (Gentra Systems, Minneapolis, MN). Human genomic DNA was prepared from cultured human skin fibroblasts using PureGene reagents and protocols (Gentra). Synthesis of MCD first strand cDNA was performed as described (22, 23) using the MCD-specific primer MCD-RT (5′-ACGGCTTAAGACCAATCATGC-3′). For analysis of the 5′ end of the MCD cDNA, the antisense oligonucleotides MCD-RACE.1 (5′-CCACCTGGCGTGTTGGTCACCGCAGG-3′) and MCD-RACE.2 (5′-GCCACCGTGAAGCTTACCAC-3′) were used in combination with the MCDstart (5′-ATGAGAGAGTGTGAGCAGG-3′) and MCD-hypoATG (5′-ATGGAGGGCTTGCGGGGACG-3′) oligonucleotides. The human heart cDNA library was obtained from Clontech. For mutation detection studies, RNA was extracted from fibroblasts derived from a severely affected malonic aciduria patient (MA002), first strand cDNA was synthesized as noted above, and two overlapping fragments of the MCD cDNA were amplified from the MA002 first strand MCD cDNA by PCR using the following oligonucleotide pairs: MCDstart and MCD-N3 (5′-CCTGCGACGCTGCTTTATG-3′), and MCD-C5 (5′-GCAATCCACCGAACATGCTG-3′) and MCD-C3 (5′-TGGGGGACAGAAGACAGTCT-3′). The resulting PCR products were sequenced directly using the same oligonucleotides used in the PCR reactions.

RESULTS

Cloning the Human MCD Gene—We previously described the development and application of context sensitive motif scanning for the in silico identification of novel peroxisomal proteins in the yeast Saccharomyces cerevisiae (24). We applied a similar approach to identify candidate peroxisomal proteins in mammalian cDNA databases from higher eukaryotes. One of the many candidates that were identified was the goose malonyl-CoA decarboxylase (A. anser MCD). This enzyme contains a perfect match to the consensus sequence for the type-1 peroxisomal targeting signal (PTS1), serine-lysine-leucineCOOH (15) and suggested that MCD might contribute to peroxisomal metabolic processes. However, it seemed that an analysis of human MCD would have greater general relevance, because there is a larger body of work on the physiological effects of malonyl-CoA in mammalian tissues (6) and because malonyl-CoA decarboxylase deficiency is associated with defects in this enzyme (10–14).

We used a computer-based approach to identify the human malonyl-CoA decarboxylase gene. The BLAST algorithm was used to scan the data base of human expressed sequence tags for cDNAs capable of encoding proteins similar to goose malonyl-CoA decarboxylase. Multiple overlapping ESTs corresponding to a single gene were identified. The cDNA clone that appeared to have the longest 5′ end was obtained from a commercial vendor and sequenced in its entirety. This clone appeared to be missing several hundred base pairs from the 5′ end. Additional MCD cDNA clones containing another 600 bp at the 5′ end were obtained from a human heart cDNA library, allowing us to assemble an apparent full-length cDNA for human MCD.

The compiled human MCD cDNA sequence (Fig. 1) is 2,121-bp long and contains a 1,362-bp open reading frame. The presumptive initiator ATG has a good match to the consensus sequence for high efficiency translation initiation (25, 26), particularly because it has purines at both the −3 and +4 positions, relative to the A of the ATG. The deduced protein product is 454-amino acids long, has a predicted molecular mass of approximately 50 kDa, and starts at exactly the same relative position as the goose cytoplasmic MCD (Fig. 2). Furthermore, it also contains the canonical PTS1 of serine-lysine-leucineCOOH. We also identified partial cDNA clones for the mouse and rat forms of MCD, and they also encode proteins that contain a PTS1 (data not shown). Additional data base searches led to the identification of the putative C. elegans MCD, which does not contain a peroxisomal targeting signal-like sequence and is considerably shorter than the vertebrate proteins at its N-terminus (Fig. 2).

Additional searches of the human EST data base identified 34 MCD cDNA clones, 4 of which were deposited only recently and contained the entire MCD ORF. However, none were as long as the MCD cDNA described in this report. 5′-RACE also failed to provide evidence for a longer MCD transcript. Knowledge of gene structure can help in the analysis of gene transcripts, and we therefore identified an MCD genomic DNA bacterial artificial chromosome clone. A fragment of this clone that hybridized to the 5′ end of the MCD cDNA clone was obtained, and sequence analysis of this clone revealed the presence of an in-frame ATG just 22-bp upstream of the 5′ end of the MCD cDNA, as well as the absence of splice acceptor sites between this ATG and the ATG we designated as the beginning of the MCD open reading frame (Fig. 3).

Any transcripts originating upstream of this ATG would have the potential to encode a longer form of human MCD, and we used PCR techniques to search for such transcripts. We prepared first strand MCD cDNA from human fibroblast mRNA and used this as template in PCR reactions containing either of two antisense MCD oligonucleotides (MCD-RACE.1 and MCD-RACE.2) and the MCD-hypoATG oligonucleotide, which spans this hypothetical upstream ATG. No detectable products were generated from these reactions even though (a) these same two combinations of primers amplified a fragment of the correct size from MCD genomic DNA and (b) the same cDNA sample and antisense oligonucleotides could be used to amplify a fragment of the correct size in PCR reactions using a 5′ primer (MCDstart), which spanned the ATG that we list at position +1 of the cDNA sequence. Similar results were obtained when these various primer pairs were used with human heart cDNA as the template. Given the sensitivity of PCR detection techniques, these data indicate that MCD transcripts containing the hypothetical upstream ATG are either of very low abundance or do not exist, at least in human fibroblasts and heart tissue.

Although we find no evidence for MCD transcripts that contain the hypothetical upstream ATG, it is useful to consider whether an mRNA that contained this sequence would encode a protein analogous to goose mitochondrial MCD. We think that this is unlikely for two reasons. First, this putative upstream ATG is followed by a pyrimidine (Fig. 3), which lessens the probability that it would serve as an efficient initiator codon were it present in a mammalian mRNA (the +4 position is almost always a purine in highly expressed transcripts (25, 26)). Even more importantly, the region between the hypothetical upstream ATG and the ATG at position +1 of the cDNA clone encodes a peptide sequence that lacks features of a mitochondrial leader sequence and shares only slight similarity to the N-terminal mitochondrial targeting signal of goose mitochondrial MCD.

As an independent test of whether the cDNA reported here is capable of encoding the full-length MCD protein we compared the mobility of endogenously synthesized MCD with that of MCD synthesized in vitro from the MCD cDNA clone. Affinity purified anti-MCD antibodies were generated and tested by immunoblot analysis of total cellular protein extracts from human fibroblasts and human hepatoblastoma cells. These antibodies detected a single polypeptide of approximately 50 kDa in both cell types (Fig. 4A), indicating that they are specific for MCD. We then synthesized MCD in vitro in a rabbit reticulocyte lysate and used immunoblot analysis to compare its mobility with that of endogenously synthesized human fibroblast MCD. A single protein was detected in each sample and their mobilities were indistinguishable from one another (Fig. 4B). Control experiments confirmed that the level of rabbit
MCD present in the in vitro translation lysates was below the limit of detection. Taken together, these various lines of evidence suggest that the MCD present in human fibroblasts corresponds to the product of the MCD cDNA clone.

Recombinant MCD Has Malonyl-CoA Decarboxylase Activity—To test the hypothesis that the gene we had identified encoded human malonyl-CoA decarboxylase, we expressed the entire human MCD protein in bacteria as a fusion with MBP. The recombinant MBP-MCD fusion protein was purified by affinity chromatography on an amylose resin. Assessment of MBP-MCD purity by SDS-polyacrylamide gel electrophoresis showed a predominant band at 90 kDa (Fig. 5), the size predicted for a fusion containing MBP (42 kDa) and human MCD (46 kDa). Purified recombinant MBP-MCD and purified MBP-LacZ (expressed and purified by the identical protocol and from the same strain of E. coli) were assayed for their ability...
convert malonyl-CoA to acetyl-CoA. MBP-MCD showed significant malonyl-CoA decarboxylase activity, with a specific activity of 3 units/mg and a $K_m$ of 220 $\mu$M for malonyl-CoA (Fig. 5).

The MBP-LacZ protein lacked activity altogether, demonstrating that the activity of the MBP-MCD fusion protein was intrinsic to the portion derived from MCD and that *E. coli* MCD does not co-purify with MBP fusion proteins on amylose resin. Although human MCD displayed a high $K_m$ for malonyl-CoA, it is only slightly higher than the $K_m$ of the goose enzyme (100 $\mu$M) (2).

**Fig. 2.** Alignment of human, goose, and worm forms of MCD. The deduced human MCD protein sequence (*Hs MCD*) is presented on the top line, the goose MCD protein sequence (*Aa MCD*) is shown in the middle line, and the putative *C. elegans* MCD protein sequence (*Ce MCD*) is presented on the bottom line. Two forms of MCD are expressed in goose, a longer form that contains a mitochondrial leader sequence and a shorter cytoplasmic form that starts at position 51 of the mitochondrial precursor but is otherwise identical. The amino acids that are present only in the precursor of the goose mitochondrial MCD are italicized. Note that the human form of MCD corresponds precisely to that of the cytoplasmic form of goose MCD.

**Fig. 3.** Genomic positions of two possible initiator codons for MCD. The nucleotide sequence presented here is from genomic DNA near the 5' end of exon 1 of the MCD gene. The 5' ends of the MCD cDNA reported here (Fig. 1) and four additional cDNA clones (identified by their GenBank accession numbers) are noted by arrows. Nucleotides of the MCD open reading frame (right side of the bottom line) are capitalized, as are the three nucleotides of the hypothetical upstream ATG. The primer that spans the hypothetical upstream ATG (*MCD-hypoATG*) and was used for reverse transcription-PCR experiments is underlined.

**MCD Is Bimodally Distributed to the Cytoplasm and to Peroxisomes**—As noted earlier, human, mouse, and rat MCD all end in a PTS1. To test whether mammalian MCD is actually associated with peroxisomes, we prepared a post-nuclear supernatant from a rat liver homogenate and then fractionated this sample by Nycodenz density gradient centrifugation. MCD was detected in perox-
Michaelis-Menten kinetics, a specific activity of 3 units/mg and a 
by coupling the MCD reaction with malate synthase and citrate synthase and assaying for NADH spectrophotometrically. MBP-MCD displayed 

Malonyl-CoA Decarboxylase Is Highly Expressed in Muscle—We also analyzed the expression of MCD mRNA in different human tissues. Multitissue Northern blots containing poly(A)⁺ RNA from 16 different tissues were hybridized with a radiolabeled MCD-specific probe (Fig. 7). A 2.3-kilobase MCD transcript was detected in all tissues that we examined. However, the abundance of the MCD mRNA appeared to vary considerably, with extremely strong expression in cardiac and skeletal muscle. The levels of MCD mRNA were much lower in liver, kidney, and pancreas, and low but detectable in all other tissues. The 2.3-kilobase size of the MCD mRNA is consistent with the 2,121-bp size of the MCD cDNA, given that the average length of the poly(A) tract is 100–200 bp. It is interesting to note that MCD expression is highest in cardiac and skeletal muscle, the two tissues that have the greatest dependence on fatty acids as an energy source and express forms of CPT1 that are extremely sensitive to inhibition by malonyl-CoA.

MCD Is Mutated in Malonyl-CoA Decarboxylase Deficiency and Maps to the Long Arm of Chromosome 16—Previous studies have reported that loss of malonyl-CoA decarboxylase activity is the cause of malonic aciduria (10–14). To test whether mutations in MCD might be responsible for loss of malonyl-CoA decarboxylase activity in these patients, we analyzed the sequence of the MCD gene from a severely affected malonic aciduria patient, MA002(14). A skin fibroblast cell line was derived from MA002, RNA was extracted from this cell line, and overlapping MCD cDNA fragments were generated by PCR using this cDNA as template. As expected, we identified a mutation in the MCD cDNA from this patient, a deletion of two T residues at position 947 and 948 of the cDNA (data not shown). This mutation is referred to as c.947–948delTT and is expected to encode a truncated protein lacking its C-terminal 142 amino acids, roughly one-third of the protein. The C-terminal third of human MCD shares significant amino acid sequence similarity with both the goose and worm forms of MCD (Fig. 2), making it extremely unlikely that a protein lacking these amino acids would retain MCD activity.

We also assessed the chromosomal location of the human MCD gene. A sequence tagged site, STS WI-11775, was generated from the 3’ untranslated region of the human MCD cDNA and localized by radiation hybrid mapping between the markers D16S422 and D16S402 on the long arm of chromosome 16. This localization of the MCD gene is consistent with the autosomal inheritance of malonic aciduria.

DISCUSSION

We recently described a computer-based search for novel peroxisomal proteins in the yeast S. cerevisiae (24). We have extended these studies to the analysis of sequence data bases of higher eukaryotes.¹ One of the candidate peroxisomal proteins that was identified in this latter search was goose malonyl-CoA decarboxylase, which contains a PTS1 at its C terminus. Loss of malonyl-CoA decarboxylase activity has been implicated in human disease (10–14), and we pursued the identification and characterization of the human MCD gene to improve our understanding of peroxisome function and to resolve the molecular basis of malonyl-CoA decarboxylase deficiency. Computer-

¹ K. A. Sacksteder and S. J. Gould, unpublished observations.

Fig. 4. Immunoblot analysis of native and recombinant MCD. A, immunoblot analysis of whole cell lysates from human skin fibroblasts (lane 1) and HepG2 cells (lane 2). B, immunoblot analysis of MCD synthesized in vitro from the MCD cDNA (lane 1) and whole cell lysates from human skin fibroblasts (lane 2).

Fig. 5. Activity of human malonyl-CoA decarboxylase. A, Coomassie stained SDS-polyacrylamide gel electrophoresis gel showing 10-kDa ladder molecular weight marker (left lane) and recombinant MBP-MCD fusion protein (right lane). B, the MBP-MCD fusion protein was assayed by coupling the MCD reaction with malate synthase and citrate synthase and assaying for NADH spectrophotometrically. MBP-MCD displayed Michaelis-Menten kinetics, a specific activity of 3 units/mg and a $K_m$ of 220 μM for malonyl-CoA.
was assayed for succinate dehydrogenase (SDH). Each gradient fraction was analyzed and a post-nuclear supernatant was subjected to differential centrifugation on a 15–40% Nycodenz gradient. The identification of the human mitochondrial enzyme (27) but its metabolic role in mitochondria has never been clearly established. The identification of human MCD has been suggested to be a mitochondrial enzyme but its metabolic role in mitochondria is currently unknown. The identification of human MCD has been suggested to be a mitochondrial enzyme but its metabolic role in mitochondria is currently unknown.

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Malonyl-CoA decarboxylase deficiency is caused by mutations in the MCD gene (22, 23). At least 19 different mutations have been described (24), 13 of which are missense mutations (25). Missense mutations create a new amino acid sequence, which is immunogenic and is likely to be a cause of malonyl-CoA decarboxylase deficiency (MCD). This has been suggested to be a mitochondrial enzyme but its metabolic role in mitochondria is currently unknown. The identification of human MCD has been suggested to be a mitochondrial enzyme but its metabolic role in mitochondria is currently unknown. The identification of human MCD has been suggested to be a mitochondrial enzyme but its metabolic role in mitochondria is currently unknown.

Malonyl-CoA decarboxylase activity and is mutated in a patient with malonyl-CoA decarboxylase deficiency (malonic aciduria), indicating that it is the human MCD gene.

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In adipogenic tissues, cytoplasmic malonyl-CoA is produced as a precursor for fatty acid synthesis. In this context, the inhibition of CPT1 by malonyl-CoA serves to prevent a futile cycle of fatty acid synthesis in the cytoplasm and fatty acid oxidation in the mitochondrion. However, the fact that acetyl-CoA carboxylase is expressed in virtually all cells, and thus, generates malonyl-CoA in many tissues that do not make fatty acids, suggests that malonyl-CoA is a general regulator of fatty acid oxidation in all tissues. This hypothesis is supported by the fact that the inhibitory effects of malonyl-CoA on CPT1 are 100 times greater in muscle than in liver (6). Not surprisingly, acetyl-CoA carboxylase is a tightly regulated enzyme and is inactivated by the AMP-dependent protein kinase when the ATP/AMP ratio falls (8, 28). This inhibitory regulation of acetyl-CoA carboxylase is thought to lower the steady-state levels of malonyl-CoA and lead to activation of CPT1 and mitochondrial fatty acid oxidation under conditions that require muscle tissue to utilize fatty acids rather than glucose as their primary energy source. Although acetyl-CoA carboxylase plays a key role in this regulatory event, the degradation of cytoplasmic malonyl-CoA is equally important. Prior studies have been unable to explain how cells rid themselves of cytoplasmic malonyl-CoA, because MCD has been thought to be an exclusively mitochondrial enzyme and because malonyl-CoA cannot cross the mitochondrial membrane. Our observation that there is a significant pool of cytoplasmic MCD suggests that this enzyme could contribute to the regulation of cytoplasmic malonyl-CoA levels and mitochondrial fatty acid oxidation. The high level of MCD expression in cardiac and skeletal muscle, tissues that require fatty acids for much of their energy and are extremely sensitive to the effects of malonyl-CoA, lends support to this hypothesis. It will be interesting to determine whether the activity of cytoplasmic MCD is regulated in a manner complementary to that of acetyl-CoA carboxylase.

The peroxisomal form of MCD is likely to play a different role in cellular metabolism than its cytoplasmic counterpart. Currently, there is no direct evidence that malonyl-CoA is produced inside peroxisomes and, thus, there is no bona fide role for MCD in peroxisomal metabolic processes. However, the β-oxidation of DFAs occurs exclusively in peroxisomes (9), and malonyl-CoA is the ultimate β-oxidation product of odd chain-length DFAs. Peroxisomal MCD may catalyze the final step in the oxidation of these compounds, converting intraperoxisomal malonyl-CoA to CO2 and acetyl-CoA. As with the acetyl-CoA that is produced by peroxisomal fatty acid β-oxidation (29), the acetyl-CoA produced by MCD could be converted to acetyl-carnitine and exported for subsequent use in the cytoplasm or mitochondria.

Although previous studies have implicated defects in MCD as the cause of malonyl-CoA decarboxylase deficiency (10–14), the identification of the MCD gene allowed us to test whether mutations in this gene are indeed the cause of this disease. We sequenced the MCD gene from a severely affected malonic aciduria patient (MA002(14)). The fact that this patient had a frameshift mutation (c.947–948delTT) that effectively deleted the C-terminal one-third of MCD strongly suggests that mutations in MCD are the cause of this disease.

The demonstration that MCD is the gene responsible for malonyl-CoA decarboxylase deficiency allows us to consider the phe-
notypes of malonyl-CoA decarboxylase deficiency patients in the context of our model for MCD function. The hypothesis that cytoplasmic MCD plays a significant role in degrading cytoplasmic malonyl-CoA suggests that its loss would result in elevated malonyl-CoA levels and inappropriate inhibition of mitochondrial fatty acid oxidation. These effects may be most severe in muscle, the tissue with greatest MCD expression, highest sensitivity of CPT1 to malonyl-CoA (6), and no other means for removing cytoplasmic malonyl-CoA. Case reports of malonic aciduria patients support this hypothesis (10–14). These patients display cardiomyopathy and diet-induced and infection-induced seizures, vomiting, hypoglycemia, and organic aciduria, an array of phenotypes that are reminiscent of mitochondrial fatty acid oxidation disorders (30). As for the peroxisomal form of MCD, we do not know how its loss may impact the phenotypes of malonyl-CoA decarboxylase deficiency patients. We might predict that loss of this enzyme would result in accumulation of dicarboxylic fatty acids. Although these patients display a marked organic aciduria, with accumulation of malonic, succinic, glutaric, suberic, and adipic acids, this organic aciduria is also a hallmark of patients with defects in mitochondrial fatty acid oxidation deficiencies. Thus, we cannot conclude from the clinical data whether there is or is not a general impairment of peroxisomal DAA oxidation. Furthermore, there is no a priori basis for concluding that a defect in peroxisomal DAA oxidation would contribute to human disease.

The clinical similarities between malonyl-CoA decarboxylase deficiency patients and patients with classical mitochondrial fatty acid oxidation disorders has been noted previously (10–14). However, the report that MCD is an exclusively mitochondrial enzyme (27) has become an accepted tenet of the field and may have precluded a coherent explanation for MCD function and dysfunction in metabolism and disease. Our localization of MCD to the cytoplasm and peroxisomes does not exclude the possibility that there may be some mitochondrial MCD but does indicate that there may be novel metabolic roles for MCD in cellular metabolism. In addition, our results have provided new insight into the molecular and metabolic basis of malonyl-CoA decarboxylase deficiency. We propose here that the phenotypes of malonic aciduria patients are chiefly caused by the loss of cytoplasmic MCD and the resulting dysregulation of mitochondrial fatty acid oxidation. Further investigations should help us determine the mechanisms by which MCD, together with acetyl-CoA carboxylase, controls steady-state levels of cytoplasmic malonyl-CoA and mitochondrial fatty acid oxidation.