MDH CUREs Community

Introduction

Metabolism, Structure, and Mechanism of Mammalian MDH

This document is intended to support faculty who are interested in MDH-related CUREs using mammalian MDH. Each reviewed publication includes a summary and limited commentary. Interpretation of collections of publications are discussed as a mini review on a particular sub-topic. This work is meant to help lower the barrier in implementing a mammalian/human MDH CURE project. A complete description of the Malate Dehydrogenase CURE Community (MCC) was published recently.

Introduction

If you are just beginning your studies of malate dehydrogenase, several excellent review articles are listed below:

Malate dehydrogenase: A model for structure, evolution and catalysis, *Goward and Nicholls*, Protein Sci. (1994) 3:1883.

Malate dehydrogenase: distribution, function and properties, *Musrati, et al.*, *Gen. Physiol. Biophys.* (1998) 17:193.

Malate dehydrogenases—structure and function, *Minárik et al.*, *Gen. Physiol. Biophys.* (2002), 21:257.

Hormone-dependent and hormone-independent control of metabolic and developmental functions of malate dehydrogenase-review, *Daniš and Farkis*, *Endocrine Regulations* (2009) 43:39.

Metabolism

Malate dehydrogenase (E.C. 1.1.1.37) is a model enzyme to study evolution, protein sorting and isoenzyme structure. The enzyme catalyzes the reaction shown in Figure 1:

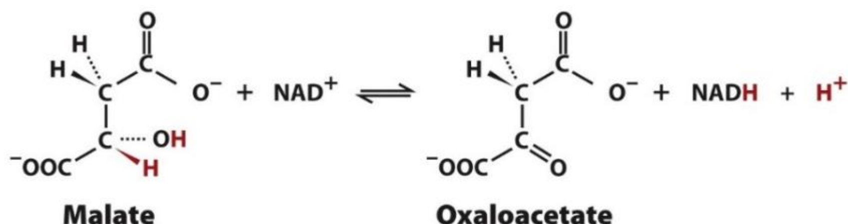

Figure 1: The MDH reaction (© by John Wiley & Sons, Inc. All rights reserved.)

In mammals, MDH exists in two isoforms, MDH1 (cytosolic) and MDH2 (mitochondrial). Two well-established roles for MDH in metabolism are:

- To catalyze the conversion of cytosolic NADH to NAD^+, providing reducing equivalents to the citric acid cycle in support of aerobic metabolism via the malate-aspartate shuttle (see Fig. 2). This function is performed by MDH1. The enzyme is highly expressed in cardiac and skeletal muscle and brain, and as such, contributes to citric acid cycle function to produce energy currency in the form of ATP to support muscle contraction, neuronal signal transmission and other cellular processes requiring ATP. The malate-aspartate shuttle is critical to maintaining the cellular NADH/NAD^+ balance, which is important for a variety of NAD^+-requiring reactions in the cell.
- To catalyze a key reaction in the citric acid cycle (also known as the Krebs' cycle or the tricarboxylic acid (TCA) cycle) in which malate is transformed to OAA; the regeneration of OAA provides one of the reactants in the first reaction of the citric acid cycle which allows for continual operation of the cycle. This function is performed by MDH2. The cycle produces NADH, fueling electron transport and generation of ATP via oxidative phosphorylation.

But both MDH1 and MDH2 are involved in more than the well-known citric acid cycle and malate-aspartate shuttle pathways. Altering the kinetics, regulation and interactions of key enzymes in these pathways can shift cellular metabolism to support various metabolic pathways including lipid synthesis and the use of glutamine as a primary carbon source for ATP production in tumor cells, and both isoforms of malate dehydrogenase play a role in these processes.

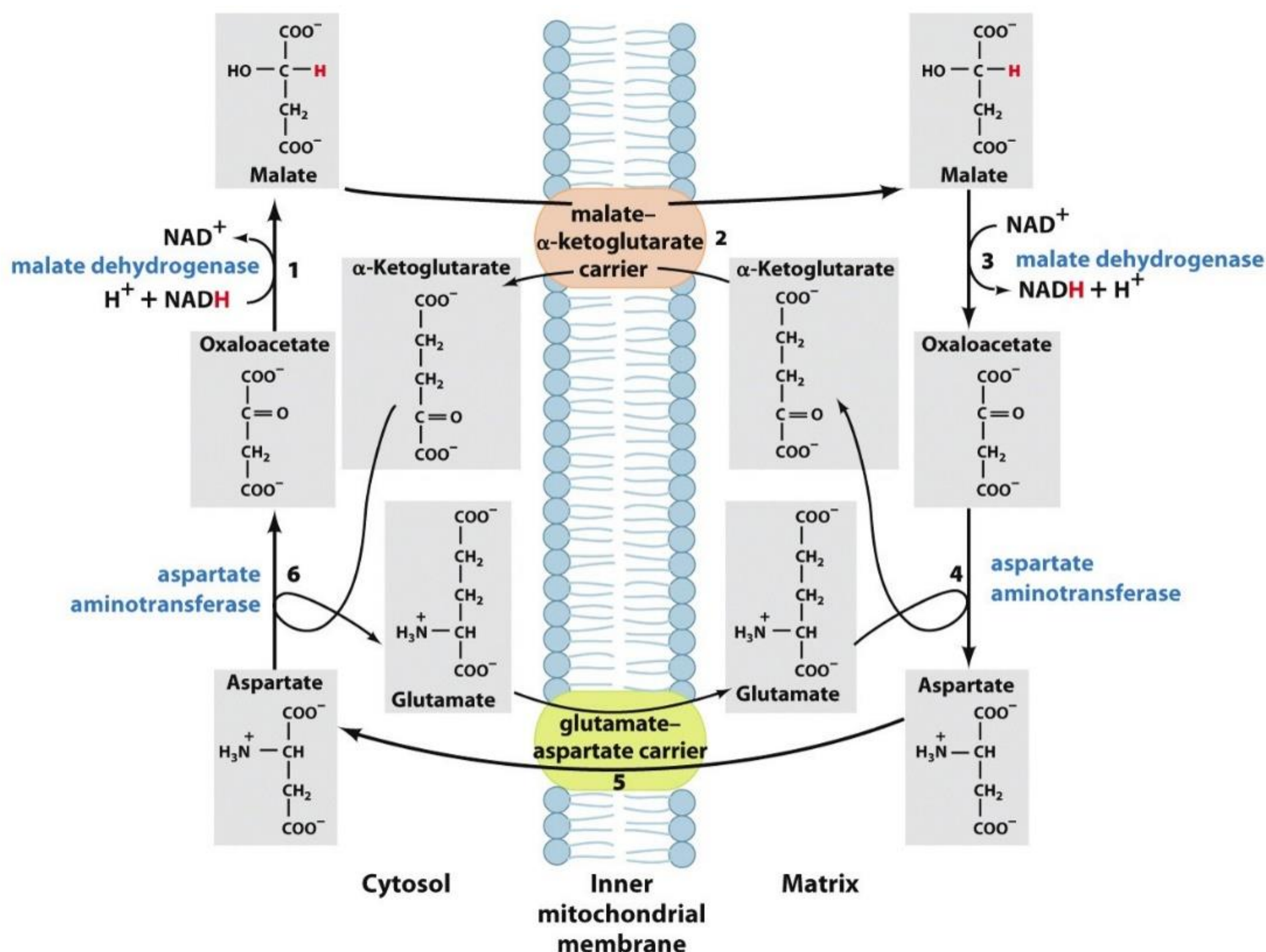

Figure 2: The malate-aspartate shuttle. The two MDH isoforms work together to transport reducing equivalents from the cytosol to the mitochondrial matrix. NADH generated as a product in the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzyme in glycolysis (not shown) must enter the mitochondrial matrix to supply reducing equivalents to the electron transport chain, but the inner mitochondrial membrane is impermeable to NADH. The problem is solved by MDH1, which catalyzes the conversion of OAA to malate in the cytosol, consuming NADH and regenerating NAD^+ in **Step 1**, providing the NAD^+ reactant for the GAPDH reaction to allow the continual operation of glycolysis. Malate produced by MDH1 from OAA is transported into the mitochondrial matrix, where it is acted upon by MDH2, which catalyzes the reverse reaction in which malate is converted to OAA. NADH enters the electron transport chain and results in the production of ATP via oxidative phosphorylation. (© by John Wiley & Sons, Inc. All rights reserved).

It is also important to consider other enzymes in metabolic reactions that have connections to malate dehydrogenase. Four cytosolic enzymes share substrates and products in common with the MDH1 reaction and are important considerations in the study of malate dehydrogenase. These include malic enzyme (ME), glutamate-oxaloacetate transaminase (GOT), ATP citrate lyase (ACL), and phosphoenolpyruvate carboxykinase (PEPCK) (see Fig. 3).

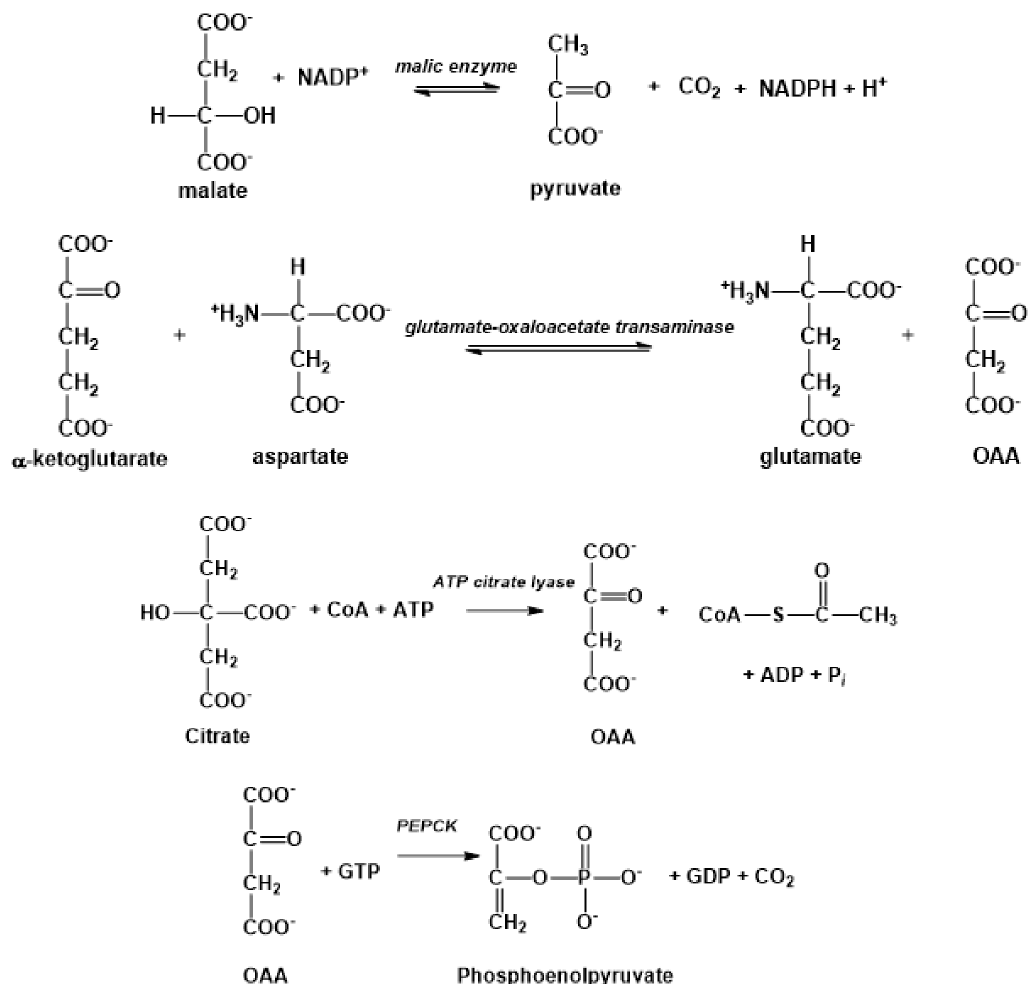

Figure 3: Reactions catalyzed by malic enzyme (ME), glutamate-oxaloacetate transaminase (GOT), ATP citrate lyase (ACL) and phosphoenolpyruvate carboxykinase (PEPCK). Synonyms, abbreviations and UniProt accession codes for these enzymes can be found in the Appendix.

Malic enzyme (ME) is found in the cytosol and is involved in lipid and other biosynthetic reactions for the purpose of regenerating NADPH. Glutamate-oxaloacetate transaminase (GOT) catalyzes a reversible transamination reaction between the α-keto acid α-ketoglutarate and the amino acid aspartate to form the amino acid glutamate and the α-keto acid OAA. ATP citrate lyase is responsible for the ATP-dependent conversion of acetyl CoA and OAA to citrate as a way to provide cytosolic acetyl CoA for fatty acid synthesis. Finally, phosphoenolpyruvate carboxykinase is one of the key enzymes of gluconeogenesis (see Fig. 4).

Note that ME, GOT and PEPCK also have mitochondrial isoforms which add to the complexity of possible research project for this and other CURE-related projects.

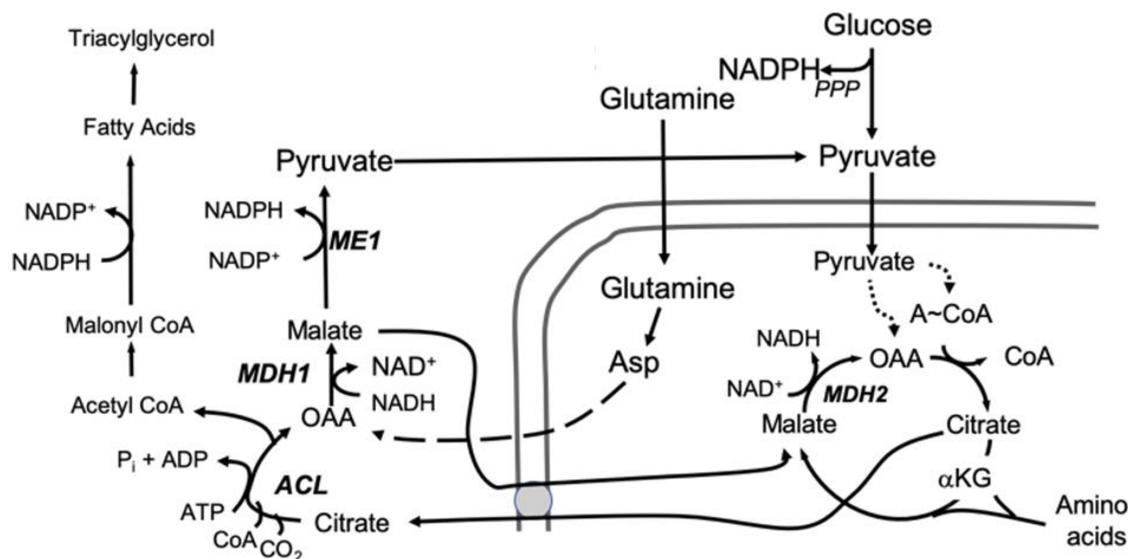

Figure 4: Reactions involving ME, GOT and ACL (see Fig. 3) and malate dehydrogenase. The GOT enzyme (not labeled specifically in this figure) is involved in the production of α-KG from amino acids in the mitochondrial matrix. This figure shows how glycolysis, the citric acid cycle, and amino acid metabolism are involved in lipogenesis.

Over the past few years the involvement of MDH in unique and tissue specific metabolism is becoming increasingly clear. New evidence suggests that the regulation of the enzyme can shift carbon flux in support of amino acid utilization in cancer cells, is involved in high metabolic demand in kidney tissues, as well as adipogenesis (see Fig. 4) and other pathways. Additional fine-tuning of MDH activity takes place by a variety of mechanisms including regulation of the production of splice variants (see **Structure** section below) as well as phosphorylation, acetylation, or methylation of the enzyme itself. Regulation of enzyme activity may involve the binding of metabolites that serve as either metabolic inhibitors or allosteric regulators that adjust the activity of the enzyme to alter carbon flux in support of cellular metabolic needs. However very little is known about many of these processes.

In glycolysis, the malate-aspartate shuttle is key to maintaining reducing equivalents in tissues lacking mitochondria, or tissues undergoing anaerobic fermentation. Under these conditions, NAD^+ regeneration for the glycolytic enzyme GAPDH is required; this is normally accomplished by LDH in which the product of glycolysis, pyruvate, is reduced to lactate using NADH as the proton donor, with concomitant production of NAD^+. MDH activity supports these dynamic metabolic processes, as the NAD^+ regenerated in the malate-aspartate shuttle in some cases is required, along with the NAD^+ regenerated by LDH, to support a proper NADH/ NAD^+ ratio. As tumor cells grow at a dysregulated pace, a high requirement for ATP is met by the Warburg effect in which glucose undergoes anaerobic fermentation to lactate even in the presence of oxygen. This is an adaptation by tumor cells where critical metabolic enzymes are expressed at higher levels and activated by a variety of post-translational modifications including phosphorylation and acetylation of the regulated enzymes. This results in an anaerobic-like metabolism to produce ATP even when oxygen, functional mitochondria, and oxidative phosphorylation processes are all present. As a result, there is a significantly higher uptake of glucose to continue the high glycolytic production of pyruvate to meet the demand for ATP, as the yield of ATP is substantially lower in anaerobic fermentation than in aerobic oxidation. Because anaerobic fermentation is less efficient than complete oxidation of glucose to carbon dioxide and water, the rate of ATP production is much higher in these cells. Depending on the tissue type, some of the glucose is shunted to biosynthetic pathways (such as the pentose phosphate pathway) producing NADPH for biosynthesis of lipids; and glucose carbons are converted to amino acids and nucleotides. Similar shifts in

intermediary carbohydrate metabolism are seen in hypoxia, both in nascent tumors where diffusion of oxygen is insufficient to support cancer cell growth, and in disease states including cardiac ischemia, brain injury.

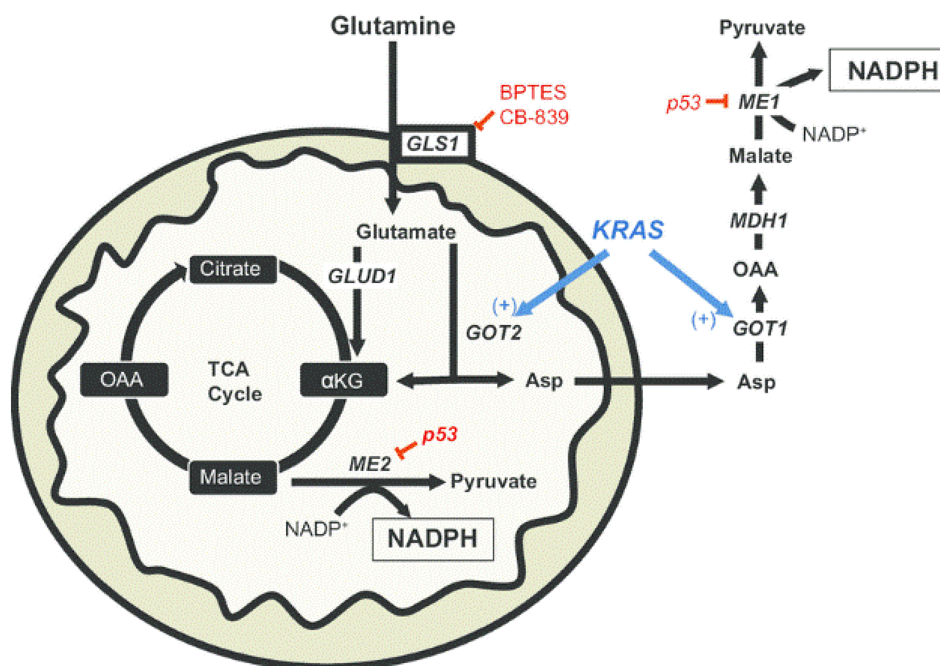

Figure 5: Reactions involving ME, GOT (see Fig. 3) and malate dehydrogenase. MDH2 (not labeled in the figure) catalyzes the conversion of malate to OAA in the citric acid cycle in the mitochondrial matrix. Here, glutamine carbons are shuttled to the mitochondrial matrix to produce ATP, and aspartate is involved in NADPH production. In normal cells, the concentration of p53 is low, but under conditions of stress, p53 levels rise, inhibiting both ME1 and ME2, and causing a shift of the activities of the cell to growth arrest, DNA repair and apoptosis. In many cancers, p53 is mutated and unable to inhibit ME1 and ME2, allowing the pathways shown here to proceed at a high rate.

Glucose alone is not enough to supply the glycolytic needs of active cells (proliferating, immune and cancer cells); thus glutamine is often used as an energy source as shown in Figure 5. Because this pathway is active in cancer cells, enzymes involved are attractive therapeutic targets. MDH is expressed at higher levels and regulated in several types of cancer cells suggesting that the enzyme is involved in more than just a “housekeeping” role. Understanding the role of MDH in directing reducing equivalents and carbohydrate metabolites is critical to developing drug treatments for various diseases.

The involvement of both MDH1 and MDH2 in adipogenesis and tumor cell energy production make these enzymes attractive to study. The dual cellular location of MDH may allow pools of enzyme to direct metabolic intermediates in divergent pathways. In our community, we focus on better understanding the structure of MDH, its kinetics and regulation, as well as how MDH interacts with other cellular proteins to support metabolism in normal and diseased states.

Structure

Malate dehydrogenase is a widely distributed enzyme in plants, animals, and bacteria. In this review, we focus on the mammalian enzyme, with particular attention paid to the human enzyme. Malate dehydrogenase belongs to the family of NAD-dependent dehydrogenases, a large well-studied family with over 100 members. Each MDH monomer consists of two different domains: The N-terminal domain contains the NAD-binding site, which contains the extended β-sheet with a connecting α-helix termed the Rossmann fold, which is characteristic of the NAD-dependent dehydrogenases. The C-terminal domain contains the substrate binding site and the active site with the amino acid side chains that participate in catalysis. The interface of the dimer consists of interacting α-helices on each monomer that bind together. Hydrophobic contacts, hydrogen bonds and water-mediated hydrogen bonds are responsible for stabilizing the dimer. In the dimeric form, the active sites on each monomer are greatly separated from one another.

In eukaryotes, there are three forms of the enzyme: a cytosolic isoform (MDH1); a mitochondrial isoform (MDH2); and a peroxisomal isoform (MDH1x). While some organisms, such as yeast, have a dedicated MDH peroxisomal gene, mammalian peroxisomal MDH is actually MDH1 whose stop codon has been read through resulting in an extended C-terminus and is shuttled to the peroxisome.

Both MDH1 and MDH2 are generally expressed as homodimers with an expected native size of about 70 kDa. While many organisms express MDH as a dimer, there are several microbial MDH enzymes that are found as monomers, some as dimers, others as tetramers, and still others that can assume multiple forms. While both mammalian enzymes catalyze the same reaction (and the catalytic residues are highly conserved), the human isoforms possess only 19% identical positions (70 residues) and 35% similar positions (119 residues) as shown by clustal alignment for the canonical MDH1 and MDH2 variants. There are several highly conserved domains between mammalian MDH1 and MDH2 isoforms including the catalytic loop. These are highlighted in the alignment shown in Figure 6.

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MDHC_HUMAN      1   --------------------MSEPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILVL   40
MDHM_HUMAN      1   MLSALARPASAALRRSFSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVS-----RLTL   55
                                         .:  :* * **:* *.  *   : *. :..      *.*

MDHC_HUMAN     41   LDITPMMGVLDGVLMELQDC-ALPLLKDVIATDKEDVAFKDLDVAILVGSMPRREGMERK   99
MDHM_HUMAN     56   YDIAHTPGVAA----DLSHIETKAAVKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTRD  111
                     **:   **      :*..  :   :*. :. ::   .:*. **.:: ..:**: ** *.

MDHC_HUMAN    100   DLLKANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTASK----SAPSIPKENFSCL  155
MDHM_HUMAN    112   DLFNTNATIVATLTAACAQHCPEAM-ICVIANPVNSTIPITAEVFKKHGVYNPNKIF-GV  169
                    **:::*..*. :  **  ::. ::: : *:.**.*:.   :::     .   *:: *  :

MDHC_HUMAN    156   TRLDHNRAKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKL--QGKEVGVYEAL  213
MDHM_HUMAN    170   TTLDIVRANTFVAELKGLDPARVNVPVIGGHAGKTIIPLISQCTPKVDFPQDQL------  223
                    * **  **:: :*   *:    *:  :* *: ..*  * :.:.. *:    .::

MDHC_HUMAN    214   KDDSWLKGEFVTTVQQRGAAVIKARKLS------SAMSAAKAICDHVRDIWF---GTPEG  264
MDHM_HUMAN    224   -------TALTGRIQEAGTEVVKAKAGAGSATLSMAYAGARFVFSLV-DAMNGKEGVVEC  275
                             :.  :*: *: *:**:  :       * :.*: : . * *      *. *

MDHC_HUMAN    265   EFVSMGVISDGNSYGVPDDLLYSFPVVIKNKTWKFVEGLP-INDFSREKMDLTAKELTEE  323
MDHM_HUMAN    276   SFVKS---------QETECTYFSTPLLLGKKGIEKNLGIGKVSSFEEKMISDAIPELKAS  326
                    .**.             :   :* *::: :*  :   *:  :..*..: :. :  **. .

MDHC_HUMAN    324   KESAFEFLSSA-                                                 334
MDHM_HUMAN    327   IKKGEDFVKTLK                                                 338
                     :.. :*:.:
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Figure 6: Clustal alignment for human MDH1 (MDHC, cytosolic) and MDH2 (MDHM, mitochondrial). The NADH binding site is shown in teal and the OAA binding site in light green, as well as the catalytic histidine for both enzymes shown in light red.

MDH1 is more polar with a higher content of acidic residues as compared to the mitochondrial MDH2 isoform. Cytosolic MDH1 contains 41 basic residues (31 Lys and 10 Arg) and 43 acidic residues (25 Asp and 18 Glu). Mitochondrial MDH2 contains 33 basic residues (25 Lys and 8 Arg) and 29 acidic residues (13 Asp and 16 Glu).

Splice variants in MDH1

There are several splice variants of cytosolic MDH all derived from a common gene. Variants differ in structure at the N-terminus. MDH monomers dimerize via interactions at the N-terminus, but otherwise, little is known about how variations in the N-terminus affect dimerization and function. The activity of the enzyme may be affected by its ability to form dimers. Degradation may take place at one of the extended cytosolic MDH isoforms; this is an observation worth further investigation. There are several MDH expressed splice variants that are not likely to have activity. Because MDH has been shown to bind to non-metabolic enzymes, it is possible that splice variants are involved in a unique form of regulation.

Note that there are several databases and websites that link to these variants. The nomenclature varies between UniProt, NCBI and others; therefore close attention must be paid to the size of the splice variant to assign the correct MDH version to its function when investigating databases and publications. As an additional clarification, the term “MDH1” refers to cytosolic MDH in humans and “MDH2” is the mitochondrial enzyme; in yeast, these terms are reversed and the MDH1 is the mitochondrial enzyme and MDH2 is the cytosolic form.

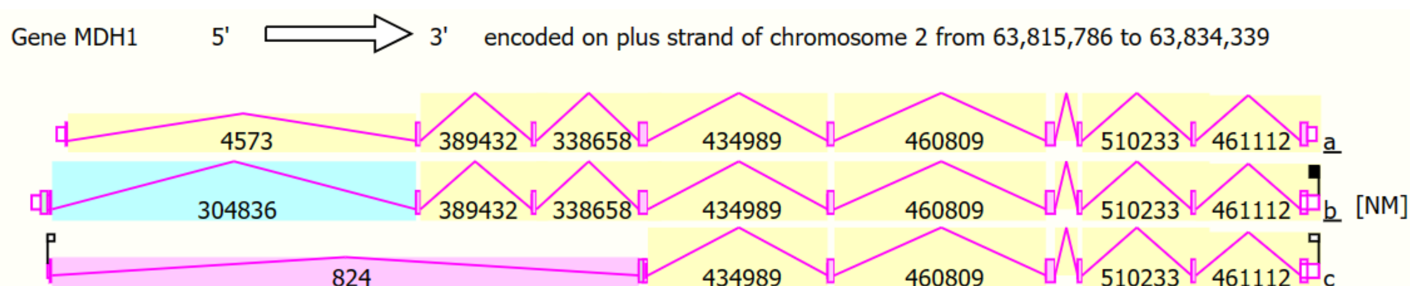

Figure 7: Shown in this figure are three of the fourteen splice variants of cytosolic human MDH1 on AceView (a curated representation of mRNA sequences from multiple public sequence databases). The AceView nomenclature is different from that of UniProt and NCBI; note that variant **a** corresponds to MDH1v3, variant **b** corresponds to MDH1v1 and variant **c** corresponds to MDH1v2 (see Table 1).

The variant names are different in various databases. For our purposes we will use the nomenclature described by UniProt. Some databases use the term “isoform” to describe splice variants of the same MDH gene. We will use the term “variants” for clarity. Three variants of cytosolic MDH1 are described in Table 1.

Note that the initial clone for bacterial expression used in the MCC was generated from a brain cDNA library and thus is the MDH1v3 variant.

Table 1: Characteristics of three MDH1 cytosolic isoforms (The Human Protein Atlas and NCBI Ace View)

	Description and expression	NCBI GenBank accession numbers			UniProt accession number and name	Structure (monomer)
		Gene	mRNA	Protein		
MDH1v1 (canonical sequence)	Expressed in all tissues; highest level in most metabolically active, i.e., brain, cardiac, adipose and others	Gene 4190	NM_001316374	NP_005908	P40925 MDHC_HUMAN P40925-1	334 amino acids 36.4 kDa
MDH1v2 (starts 89 residues after N-terminus of v1)	Shorter than v1 as it lacks the 5' exon with an in-frame downstream start codon, expressed in skin and fibroblasts		NM_001199112.2 Referred to as "isoform 3" on NCBI	NP_001186041.1 Referred to as "isoform 3" on NCBI	P40925 MDHC_HUMAN P40925-2	245 amino acids 27.0 kDa
MDH1v3	A longer variant, it contains an alternative start codon N terminal from variant 1, expressed in various regions of the brain		NM_001199112.2 Referred to as "isoform 2" on NCBI	NP_001186040.1 Referred to as "isoform 2" on NCBI	P40925 MDHC_HUMAN P40925-3	352 amino acids (18 additional N-terminal amino acids) 38.6 kDa

It should be noted that MDH1v2 is missing some of the NADH/NAD^+ binding domain and may not be active, while the longer, variant 3 (MDH1v3) has a unique phosphorylation site, that in yeast, targets the protein when phosphorylated for degradation.

These are the three (four if one includes the peroxisomal C-terminal extended MDH) variants identified on both NCBI and UniProt, and based on RNA expression data bases, are found in multiple tissues/cell types. There are however many other splice variants that have been catalogued on various databases. The Human Protein Atlas identifies nine different variants, many too small to be active, but expressed in some tissues. NCBI's Ace View shows fourteen variants. Several recent publications also show that microRNA can impact the expression and potentially splice variants of MDH that we will not cover here. Thus the final understanding of MDH expression has yet to be fully understood.

Splice variants in MDH2

Like MDH1 there are a few different variants of the mitochondrial MDH2, however the canonical variant, MDH2v1 is the predominant version of mitochondrial MDH in mammals. Several shorter variants exist including a variant with the same N- and C-termini but missing an in-frame exon (MDH2v2) and a variant with a later alternative start codon which produces a protein with a shortened N-terminus (MDH2v3) (see Fig.8). AceView shows there are six total identified splice variants, but only variant 1 has the active site domain and is the overwhelming predominant transcript across tissues. We are using the canonical MDH2v1version in the MCC and for simplicity purposes it will be referred to as MDH2.

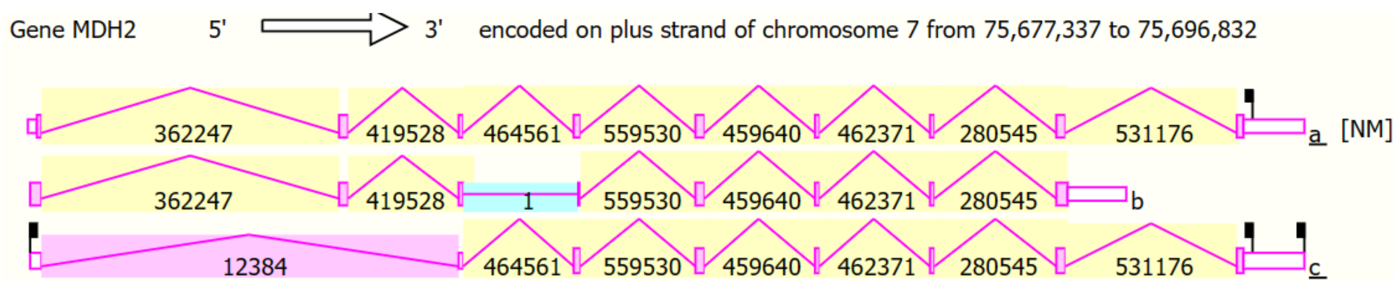

Figure 8: Shown in this figure are three of the six splice variants of mitochondrial human MDH2 on AceView. The AceView nomenclature is different from that of UniProt and NCBI; note that variant **a** corresponds to MDH2v1, variant **b** corresponds to MDH2v2 and variant **c** corresponds to MDH2v23 (see Table 2).

Table 2: Characteristics of three MDH2 cytosolic isoforms (The Human Protein Atlas and NCBI Ace View)

	Description and expression	NCBI GenBank accession numbers			UniProt accession number and name	Structure
		Gene	mRNA	Protein		
MDH2v1 (canonical sequence)	Expressed in all cells/tissues with mitochondria, found at the highest levels in heart followed by gut, kidney and adrenal tissues	NCBI Gene ID 4191	NM_005918	NP_005909.2	P40926 MDHM_HUMAN P40926-1	338 amino acids 35.5 kDa
MDH2v2	Same N and C termini but missing in-frame exon			NP_001269332.1	P40926 MDHM_HUMAN P40926-2	296 amino acids
MDH2v3	Later alternative start codon resulting in a shortened N-terminus			NP_001269333.1	P40926 MDHM_HUMAN P40926-3	231 amino acids

MDH1 structure

The structure of human cytosolic MDH1 had not been published at the time of the inception of the MCC. However, it was noted that there is a high homology between human and porcine MDH1 and as such, the enzymes could be assumed to have identical three-dimensional structures. The porcine MDH1 structure (4MDH) and structures with substrate mimics (OAA and NADH analogs) bound in the active site (5MDH) have been published in the Protein Data Bank

Table 3: Structures of porcine MDH1 in the Protein Data Bank. "MAK" is α-ketomalonic acid, an OAA analog.

Species	Entry	Align	Ligands	Other Ligands	Chains	Res. (A)	Length
Sus scrofa	4MDH	/	NAD	SO4	A/B	2.5	1-334
Sus scrofa	5MDH	/	NAD	MAK	A/B	2.4	1-333

Note: Using the website/software Phyre2, we have a built a predicted structure of apo human MDH1 splice variant 1 to share with the MCC.

Recently the crystal structure of MDH1 was determined by McCue and Finzel and is shown in Figure 9. The research team noted that the crystal structure of porcine MDH1 was determined 25 years ago but noting that MDH1 is overexpressed in cancer cells, and is emerging as a therapeutic target, interest was renewed in the study of the human MDH1. The PDB accession code is 7RM9. The protein was crystallized with malonate bound to the active site. The human structure, as expected, was found to be very similar to the porcine structure (as the two share 95% amino acid identity), but because the human structure was crystallized without NAD^+, the investigators were able to obtain some insights on how NAD^+ binds to the enzyme. They suggest that the β4- α4 loop in which the NAD^+ binds has greater conformational flexibility that was initially believed. This is interesting because the additional flexibility may pave the way for the design of NAD^+ analogs that can bind to the site as competitive inhibitors.

The authors also compared their MDH1 structure with the structure of MDH2 (PDB: 2DFD) and found slight differences in the substrate binding sites of the two enzymes which would potentially allow the development of therapeutic agents that could inhibit one isoform and not the other.

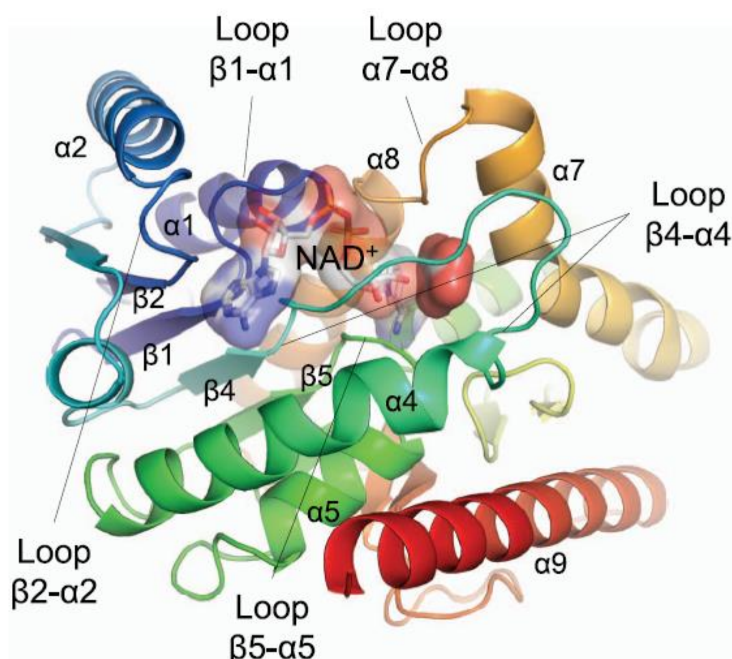

Figure 9: Crystal structure of human MDH1. NAD^+ is shown as sticks and the substrate analog malonate is shown as a space-filling model. The β4-α4 loop (shown in teal) is a flexible loop that closes over the substrates when they bind to the enzyme, protecting the substrates from the aqueous solvent (from McCue and Finzel © by the authors, published by the American Chemical Society. All rights reserved).

MDH2 structure

The structure of human MDH2 has been published, with substrates and inhibitors bound (see UniProt or PDB database). Table 4 displays the current available structures for mammalian MDH2 with various ligands and other information.

Table 4: Structures of human MDH2 enzymes deposited in the Protein Data Bank

Species	Entry	Align	Ligands	Other Ligands	Chains	Res. (A)	Length
Homo sapiens	2DFD	/	NAD	HIS, MLT, ALA, CL	A/B/C/D	1.90	20-338
Homo sapiens	4WLE	/	CIT	/	A/B/C/D	1.90	20-338
Homo sapiens	4WLF	/	LMR	PO4	A/B/C/D	2.20	20-338
Homo sapiens	4WLN	/	APO	PO4	A/B/C/D	2.28	20-338
Homo sapiens	4WLO	/	OAA	NAI	A/B/C/D	2.50	20-338
Homo sapiens	4WLU	/	NAD	LMR	A/B/C/D	2.14	20-338
Homo sapiens	4WLV	/	NAD	PO4	A/B/C/D	2.40	20-338
Sus scrofa	1MLD	94.08%	CIT	/	A/B/C/D	1.83	25-338
S. cerevisiae	1HR9	51.60%	APO	EPE, ZN	O/P/Q/R	3.01	18-334

Mechanism

Malate dehydrogenase is similar to lactate dehydrogenase (LDH) in that both enzymes catalyze the reduction of an α-hydroxyacid to its corresponding α-keto acid, with NADH serving as the electron donor. For malate dehydrogenase, the α-hydroxy acid and α-keto acid are malate and OAA, respectively; for LDH, the α-hydroxy acid is lactate and the α-keto acid is pyruvate. Both enzymes catalyze reversible reactions. The malate → OAA conversion proceeds via an ordered bi-bi reaction in which the pyridine nucleotide coenzyme binds first, followed by the dicarboxylate substrate. Upon formation of the ternary complex a flexible loop closes over the substrates, protecting the active site from the aqueous medium. The enzyme's active site consists of a hydrophobic cleft with a hydrogen-bond linked histidine-aspartate dyad that likely acts as a proton relay in the catalytic cycle. The flexible loop (residues 105-123 in MDH1v3 and 87-105 in MDH2) is highly conserved among MDH isoforms.

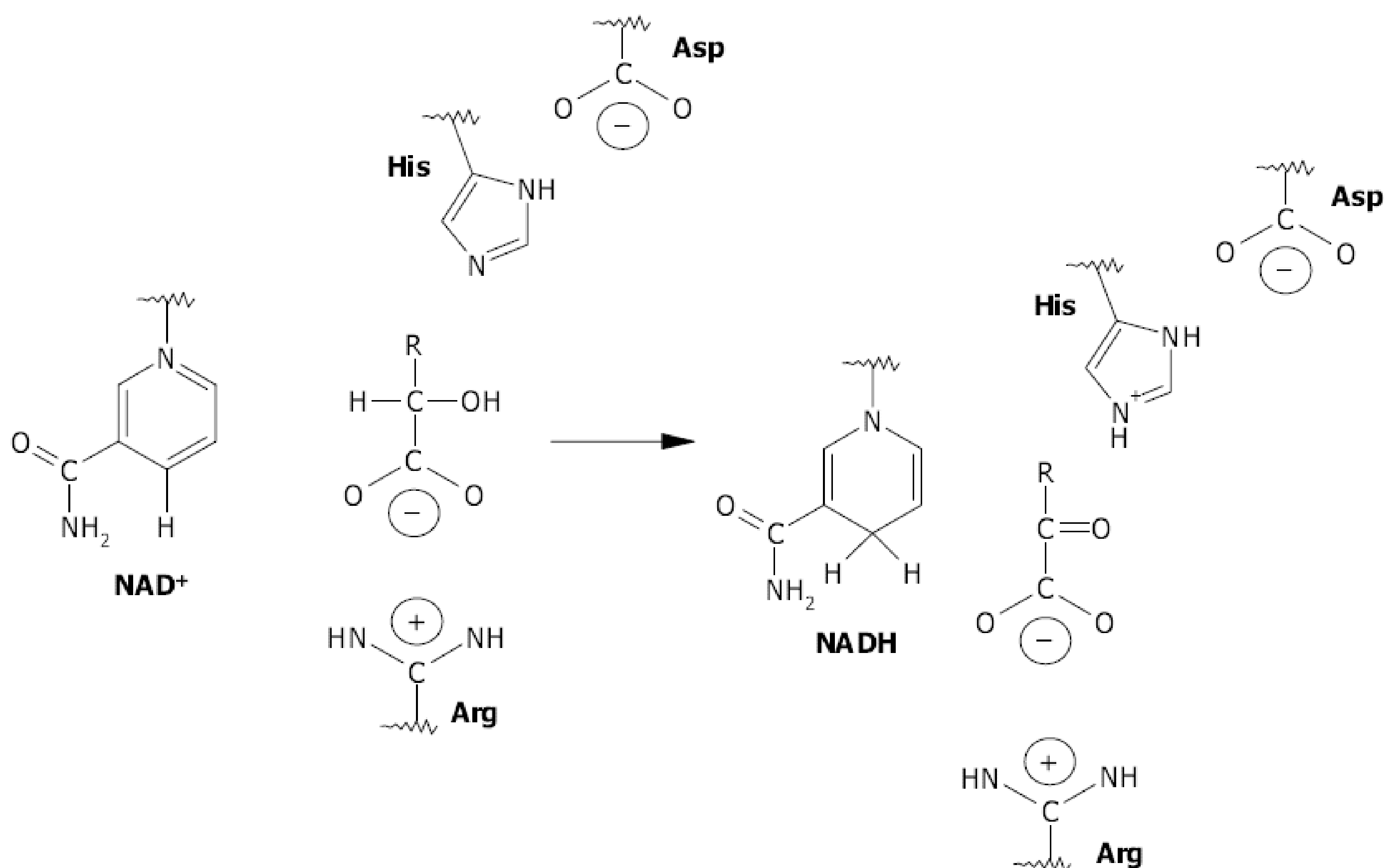

Figure 9: Catalytic mechanism of MDH. The active site consists of a His-Asp dyad that likely serves as a proton relay during catalysis. The guanidino group side chain of an Arg residue in the active site assists in anchoring the substrate to the active site by interacting with the negatively charged carboxylase group (© *Gen. Physiol Biophys.* All rights reserved).

Literature review

Malic dehydrogenase. VII. The catalytic mechanism and possible role of identical protein subunits, *Harada and Wolfe,* [J. Biol. Chem. (1968) 243: 4131]

Using mitochondrial pig heart MDH2, the impact of an OAA mimic and competitive inhibitor, ketomalonate, was studied. This study, like other work conducted by this research team, focuses on the inhibition of product/substrate as it pertains to the binding/reacting mechanism: The nucleotide binds first, followed by the substrate; at the conclusion of the reaction the oxidized substrate is released first followed by the reduced nucleotide. They argue that studies using this competitive inhibitor reveal a different binding mechanism involving two substrate binding sites on dimeric MDH. They suggest that either OAA or nucleotide binding to one of the dimers impacts the activity of the corresponding monomer. They even suggest that one of the dimers is a regulator for the second monomer and this cycles back and forth in which each monomer is functional for one cycle and the other is regulatory, and in the following cycle the monomers swap roles. This is the first suggestion that one monomer pair of the dimer could be inhibited by OAA.

Commentary: To confirm their hypothesis, a mutant MDH (which doesn't bind OAA but does bind citrate or ketomalonate) MDH could be separated into monomers, and refolded with active wild-type MDH. The kinetics and the structure of this heterodimer could be studied. It would be interesting to investigate both MDH1 and MDH2 to determine if the effect of OAA is specific to MDH2. Activity at a variety of pH values would also be interesting to investigate. How could this phenomenon be explained—does pH impact the binding of OAA and/or citrate, or is the explanation for this observation due to changes at the subunit interface?

Characterization of the kinetics of cardiac cytosolic malate dehydrogenase and comparative analysis of cytosolic and mitochondrial isoforms, Dasika, et al., [Biophys J. (2015) 108:420].

A very comprehensive study which further established the mechanism and pH dependence of the activities of both cardiac cytosolic and mitochondrial MDH. The ordered mechanism for both isoforms is an ordered bi-bi where nucleotide (NADH or NAD^+) binds followed by OAA or malate, and after the proton transfer, the active loop closes on the binding site. Under physiological conditions the mitochondrial MDH activity is predicted to be higher than cytosolic enzyme. Interestingly at low concentrations of malate (5-150 μM) both cytosolic and mitochondrial MDH have the same activity in the malate → OAA direction; however at higher malate concentrations, the mitochondrial isoform is more active. In the OAA → malate direction at low NADH concentration (~5 μM), cytosolic MDH activity is higher and at higher NADH concentration mitochondrial activity is higher. These observations are consistent with the physiological roles and cellular locations of both enzymes, and the concentrations of metabolites found in cells in vivo. The pH dependency of both isoforms follows similar patterns. At mitochondrial physiological conditions at pH 6.5 both isoforms are relatively inactive, however as the pH rises to pH 7.5 and 8, both activities increase, with the mitochondrial MDH activity showing a greater increase at 2-3 times greater than the cytosolic enzyme. Under cytosolic conditions, the rate of MDH1 is greater at all pHs compared to MDH2, suggesting ideal behavior for each isoform under physiological conditions. It is interesting that in the malate → OAA direction, in the presence of millimolar amounts of NAD^+ and malate, increasing pH leads to increasing activity up to pH 9 for both enzymes. But at micromolar concentrations, for the reaction in the OAA → malate direction, the activity is significantly higher at lower pH (6.5 – 7.5) and the activity continue to drop significantly at pH 8.5 and higher.

Commentary: When comparing enzyme assay results in published papers, or among MCC member institutions, it's important to consider the pH at which the enzyme assays were conducted.

Appendix

Table A1: Cellular enzymes important in the study of MDH1 and MDH2

Enzyme name	Abbreviation	Alternative names or abbreviations	Cellular location	UniProt ID	UniProt accession number
NADP dependent malic enzyme	ME1		Cytosol	MAOX_HUMAN	P48163
Glutamate oxaloacetate transaminase	GOT1	aspartate transaminase (cAST, AST1) aspartate aminotransferase (cAAT, AAT1)		AATC_HUMAN	P17174
ATP citrate lyase	ACL		Cytosol	ACLY_HUMAN	P53396
Phosphoenol-pyruvate carboxykinase	PCK1, cPEPCK		Cytosol	PCKGC_HUMAN	P35558
Citrate synthase	CS		Mitochondrion	CISY_HUMAN	O75390
Glutamate oxidase transaminase	GOT2	aspartate transaminase (mAST, AST2) aspartate aminotransferase (mAAT and AAT2)	Mitochondrion	AATM_HUMAN	P00505
NAD dependent malic enzyme	ME2		Mitochondrion	MAOM_HUMAN	P23368
NADP dependent malic enzyme	ME3		Mitochondrion	MAON_HUMAN	Q16798
Fumarase	FH		Mitochondrion	FUMH_HUMAN	P07954
Aconitase	ACO2		Mitochondrion	ACON_HUMAN	Q99798
Phosphoenol-pyruvate carboxykinase	PCK2, mPEPCK		Mitochondrion	PCKGM_HUMAN	Q16822