Expression and Function of a Mislocalized Form of Peroxisomal Malate Dehydrogenase (MDH3) in Yeast*

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The malate dehydrogenase isozyme MDH3 of Saccharomyces cerevisiae was found to be localized to peroxisomes by cellular fractionation and density gradient centrifugation. However, unlike other yeast peroxisomal enzymes that function in the glyoxylate pathway, MDH3 was found to be refractory to catabolite inactivation, i.e. to rapid inactivation and degradation following glucose addition. To examine the structural requirements for organellar localization, the Ser-Lys-Leu carboxyl-terminal tripeptide, a common motif for localization of peroxisomal proteins, was removed by mutagenesis of the MDH3 gene. This resulted in cytosolic localization of MDH3 in yeast transformants. To examine structural requirements for catabolite inactivation, a 12-residue amino-terminal extension from the yeast cytosolic MDH2 isozyme was added to the amino termini of the peroxisomal and mislocalized “cytosolic” forms of MDH3. This extension was previously shown to be essential for catabolite inactivation of MDH2 but failed to confer this property to MDH3. The mislocalized cytosolic forms of MDH3 were found to be catalytically active and competent for metabolic functions normally provided by MDH2.

Differentially compartmentalized isozymes of malate dehydrogenase in eucaryotic cells catalyze the NAD(H)-dependent interconversion of oxaloacetate and malate. In mammalian cells this reaction, catalyzed by mitochondrial and cytosolic isozymes, respectively, is a critical step in the tricarboxylic acid cycle and in gluconeogenesis. The two isozymes also participate in the malate/aspartate shuttle cycle, a mechanism for exchange of reducing equivalents between cellular compartments. In yeast and plant cells, a third isozyme localized in peroxisomes catalyzes a step in the glyoxylate pathway. This pathway allows formation of C4 metabolites from C2 precursors. The malate dehydrogenase isozyme family is therefore an ideal focus for analysis of structural features responsible for differential compartmentation and metabolic function.

To initiate molecular genetic studies, the three isozymes of malate dehydrogenase have been purified from Saccharomyces cerevisiae, and the corresponding genes have been cloned, sequenced, and disrupted (1–3). The isozymes are all homodimers, and they exhibit similar kinetic properties (3). The aligned amino acid sequences have residue identities ranging from 43 to 50%. Among conserved residues are those with catalytic functions or those that participate in cofactor binding (4, 5). Salient differences include regions with putative functions in organellar targeting. The yeast mitochondrial isozyme (MDH1, subunit molecular weight = 33,500), for example, has a 17-residue amino-terminal extension not present on the other isozymes; this extension is removed upon mitochondrial import (6). The peroxisomal isozyme (MDH3, subunit molecular weight = 37,200) has a unique carboxyl-terminal tripeptide sequence, Ser-Lys-Leu. Similar SKL termini on other peroxisomal proteins have been found to be necessary and sufficient for organelar localization (7, 8). The importance of this carboxyl terminus for localization of MDH3 is a focus of this work.

Cellular levels of all three malate dehydrogenase isozymes are low in yeast cells cultivated with glucose as a carbon source (2, 3, 9). For MDH1, this appears to be the result of glucose repression of gene expression as is the case for many mitochondrial proteins. Levels of MDH2, the cytosolic isozyme (subunit molecular weight = 40,700), exhibit more dramatic changes. This enzyme, along with others with functions in gluconeogenesis or in the glyoxylate pathway in yeast (10–12), is subject to rapid glucose-induced inactivation and degradation (13), a phenomenon termed catabolite inactivation. MDH2 has a 12-residue amino-terminal extension not present on aligned sequences of mature MDH1 or of MDH3 polypeptides, and we have shown that removal of this amino-terminal extension makes MDH2 refractory to catabolite inactivation (13). In this report, we investigate changes in MDH3 expression with glucose addition and test the sufficiency of the MDH2 amino-terminal extension for catabolite inactivation.

Disruption of the chromosomal MDH loci in yeast and analysis of resulting growth phenotypes have elucidated metabolic functions of each isozyme. Disruption of the MDH1 gene in a haploid strain produces an inability to grow with acetate as a carbon source (9). A similar growth phenotype is obtained with disruption of genes encoding several other tricarboxylic acid cycle enzymes including citrate synthase (14) and NAD-dependent isocitrate dehydrogenase (15) and is attributed to an energy deficiency with this carbon source. Disruption of the MDH2 gene produces an inability to grow with acetate or ethanol on minimal medium (2). Since such a mutant grows with C2 carbon sources on rich medium, this phenotype indicates an auxotrophic requirement, perhaps for C2 metabolites. Singh et al. (16) have reported that disruption of MDH3 eliminates growth with oleate as a carbon source, perhaps indicative of a deleterious effect on peroxisomal β-oxidation. These different phenotypes imply distinct and critical metabolic functions for each isozyme.

Because of the unfavorable equilibrium for formation of malate from oxaloacetate (ΔG°r ≈ +7 kca/mol), it has been proposed that malate dehydrogenase may physically associate with other specific enzymes within a metabolic pathway to
ensure direct transfer of oxaloacetate (17). One of our goals in manipulating the yeast MDH isozymes is to assess the ability of isozymes, which catalyze the same reaction but which have structural dissimilarities, to function in alternative metabolic pathways and cellular compartments. In this report, we examine the ability of MDH3, normally found in peroxisomes, to function in the cytosol.

**EXPERIMENTAL PROCEDURES**

**Growth Conditions and Yeast Strains**—Yeast strains were cultivated in rich YP medium (1% yeast extract, 2% bactopectone) or in minimal YNB medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, pH 6.0) containing supplements of 20 μg/ml to supplement auxotrophic requirements for growth. The carbon source added to 2% was glucose, ethanol, or acetate. Growth rates in liquid cultures were monitored spectrophotometrically as absorbance at 600 nm. For glucose inactivation studies, strains were cultivated in YP medium plus 2% acetate and then transferred to YP medium containing 5% glucose as described previously (13). For cellular fractionation studies, yeast strains were precultivated in YNB medium containing 2% raffinose and then transferred to induction medium (YP medium with 2% ethanol and 0.1% oleate) and grown for at least 24 h to an A600nm of 1.0.

Yeast strains used in this study were the parental haploid strain S173–6B (MATa leu2–3, 112 his3–1 ura3–52 trpl–289; Ref. 18) and a collection of mutants derived from this strain containing disruption mutations in MDH loci. The collection was obtained by a genetic cross of a haploid strain containing deletion/HIS3 insertion mutations in MDH1 and MDH2 chromosomal genes (1, 2) with a haploid strain of the opposite mating type containing a deletion/LEU2 insertion mutation in the MDH3 gene (3). Haploid strains obtained following tetrad dissection were identified using protein immunodots and Southern blots as described previously (1–3) to define segregation patterns for the disrupted loci. A complete collection of MDH strains was obtained; this includes the wild type, strains containing each single gene disruption, strains containing the three possible combinations of double disruptions, and a strain containing the triple gene disruption. The method of Ito et al. (19) was used for yeast transformations.

**Plasmid Construction and Mutagenesis**—Plasmid DNA isolation, subcloning, and bacterial transformations into Escherichia coli strain DH5α (20) were conducted as described by Maniatis et al. (20).

The yeast MDH3 gene on a 3.5-klb pair HindIII restriction fragment was previously subcloned into a Bluescribe plasmid (designated pBSMDH3; Ref. 3). This plasmid was used to prepare single-stranded DNA for oligonucleotidedirected mutagenesis. A 66-mer oligonucleotide (5′-GAATTCGCGACTTTGACCGAATCTTGTTCTATGGA-TGTTGTAACGAGTGGACAGCTTGTATGATTG-3′) was used as complementary to the 5′-coding region of MDH3 was used for “loop-in” mutagenesis to insert 12 codons from the amino-terminal coding region of MDH2 between the AUG translational initiation codon and the codons for the amino terminus of MDH3. The resulting plasmid was designated pBSMDH2/3. The Bluescribe plasmids, pBSMDH3 and pBSMDH2/3, were used for subsequent mutagenesis to delete three codons (Ser-Lys-Leu) from the carboxyl-terminal end of the MDH3 coding region. This was accomplished using a 20-mer oligonucleotide (5′-AATT-GAAGTAGCTAAGAGTCTAGGAG-3′) for “loop-out” mutagenesis. The resulting plasmids were designated pBSMDH3ΔSKL and pBSMDH2/3ΔSKL.

Oligonucleotide-directed mutagenesis was conducted as described by Zoller and Smith (21). Bacterial transformants containing the altered sequences were identified by differential colony hybridization using as probes appropriate oligonucleotides, which were 5′-end-labeled with 32P using polynucleotide kinase. Single-stranded DNA was prepared from bacterial colonies, and mutations were confirmed by nucleotide sequence analysis. The dideoxy method (22) was used for sequence analysis with oligonucleotide primers previously used to sequence the MDH3 gene (3). Following confirmation of nucleotide sequences, the MDH3 gene constructs on 3.5-klb pair HindIII fragments were cloned into plasmid SKL, a yeast-bacterial shuttle plasmid containing a centromere cassette for single-copy expression in yeast and a URA3 gene for selection (23). The resulting plasmids were designated pRSMDH3, pRSMDH3ΔSKL, pRSMDH2/3, and pRSMDH2/3ΔSKL.

**Cellular Fractionation and Protein Analyses**—For cellular fractionation, yeast cells were lysed, and cellular extracts were prepared by Dounce homogenization as described by Daum et al. (24). Soluble cytosolic fraction and organellar pellets were obtained by centrifugation at 12,000 × g for 20 min. The pellets were resuspended and layered, without additional washing to preserve peroxisomal integrity, onto 20 ml of 15–37% linear Nycodenz gradients (25). The gradients were centrifuged at 4 °C for 90 min at 36,000 rpm in a Beckman 50.2 Ti rotor. One-ml fractions were collected for enzyme assays and immunoblot analysis.

Spectrophotometric enzyme assays for malate dehydrogenase (9) and catalase (27) were conducted as described previously. Protein concentrations were determined by the Bradford method (28) using bovine serum albumin as the standard. Immunoblot analyses were performed using polyclonal antiserum against MDH1 (9), MDH2 (2), MDH3 (3), and mitochondrial NADP-specific isocitrate dehydrogenase (IDH; Ref. 26), and for enzyme assays (C) for malate dehydrogenase and catalase. Activities are expressed relative to peaks detected within the gradient. The large increase in malate dehydrogenase activity in fractions at the top of the gradient is due to the abundance of mitochondrial MDH1.

**RESULTS**

**Peroxisomal Localization and Expression of MDH3**—Previous studies established that the MDH3 isozyme is enriched in an organelar pellet following cellular fractionation of lysed yeast cells (3). As illustrated in Fig. 1A, all three malate dehydrogenase isozymes are immunochemically detectable and electrophoretically distinguishable in a whole cell lysate (lanes H) from our parental yeast strain cultivated with ethanol and oleate as carbon sources; this condition induces expression of
the malate dehydrogenases (29) and of catalase (30). MDH1
and MDH3, but not MDH2, are immunochemically detectable
in an organellar pellet (lanes P) obtained as described under
“Experimental Procedures.” MDH2 is primarily found in the
soluble cytosolic fraction (lanes S), which also contains detect-
able levels of MDH1 and MDH3, presumably due to organellar
breakage during homogenization. To further separatemito-
chondrial and peroxisomal organelles, we have adapted the
technique for Nycodenz gradient centrifugation described by
Singh et al. (25). Under the conditions of centrifugation of the
organellar pellet used here, peroxisomes, with catalase activity
as a marker, are enriched in fractions within the Nycodenz
gradient (Fig. 1C, fractions 8–14), whereas mitochondria, with
NADP-dependent isocitrate dehydrogenase as an immuno-
chemical marker (26), remain at the top of the gradient (Fig.
1B, fraction 18). Immunoblot analysis demonstrates an enrich-
ment of MDH3 in peroxisomal fractions and of MDH1 in mito-
chondrial fractions (Fig. 1B). Based on relative levels of activity
in these and similar fractionation experiments conducted with
parental and various MDH gene disruption strains, MDH3
accounts for approximately 10% of the total cellular malate
dehydrogenase activity under these growth conditions.

Most yeast peroxisomal enzymes that function in the glyox-
ylate pathway, including malate synthase (11) and isocitrate
lyase (12), are subject to rapid glucose-induced inactivation and
degradation. We therefore investigated this phenomenon for
MDH3. The parental strain was cultivated with acetate as a
carbon source and then shifted to glucose medium as described
under “Experimental Procedures.” As shown in Fig. 2 and as
previously reported (13), levels of MDH2 in whole cell extracts
are rapidly depleted after glucose addition and are immuno-
chemically undetectable after 60 min, whereas MDH1 levels
are gradually diminished due to glucose repression of expres-
sion. Changes in levels of MDH3 in the same extracts are seen
to more closely resemble those for MDH1 (also cf. Fig. 6A) and
not for MDH2, suggesting that MDH3 is subject to glucose
repression but is not subject to the phenomenon of rapid glu-
cose-induced degradation. With steady state growth conditions,
we have also found that immunochemical levels of MDH1 and
MDH3 are detectable in glucose grown cells at levels 5-10-fold
below acetate growth levels (3, 6), whereas MDH2 is not immu-
nochemically detectable in extracts from glucose-grown
cells.

Construction and Expression of Mutant Forms of MDH3—To
gain an understanding of the roles of compartmental localiza-
tion and structure that contribute to the different effects of
-glucose on MDH3 and MDH2 expression, we constructed mu-
tant forms of MDH3 based on sequence differences between the
two isozymes illustrated in Fig. 3. Among the major structural
differences is an amino-terminal extension of 12 amino acid
residues on MDH2, which is not present on the aligned se-
quences for MDH3 and the mature form of MDH1. In a previ-
ous study, we constructed a mutant form of MDH2 lacking this
12-residue extension. The truncated enzyme was found to be
active but no longer subject to catabolite inactivation (13).
Another obvious structural difference is the carboxyl-terminal
SKL tripeptide unique to MDH3. As mentioned above, this and
similar tripeptides have been demonstrated to be necessary
and sufficient for organellar localization of several peroxisomal
proteins (7, 8).

To assess the importance of these structural features for
localization and turnover, the cloned MDH3 gene was used for
oligonucleotide-directed mutagenesis as detailed under “Exper-
nmental Procedures” to “loop in” codons for the MDH2 amino-
terminal extension and to “loop out” codons for the carboxy-
terminal SKL tripeptide. Four variants of the MDH3 gene were
constructed and cloned into pRS316 (23), a centromere-based
shuttle plasmid carrying the yeast URA3 gene for selection. As
shown in Fig. 4, these variants encoded authentic MDH3
(MDH3) (A), MDH3 lacking the carboxyl-terminal SKL
(MDH3ΔSKL) (B), MDH3 with the MDH2 amino-terminal ex-
tension (MDH2/3) (C), and MDH3 with the MDH2 extension
and lacking the carboxyl-terminal SKL (MDH2/3ΔSKL) (D).

For different experiments described in following sections, the
plasmids carrying wild type and mutant forms of the MDH3
gene were transformed into haploid yeast strains containing
various disruptions in the three MDH genomic loci. Construction
of a complete collection of yeast MDH mutants by a genetic
cross is described under “Experimental Procedures.”

For initial tests of expression, a strain carrying genomic
disruptions in all three MDH loci was used for transformation
with the pRS plasmids. Ura+ transformants were isolated and
grown on rich medium with ethanol as the carbon source; this
carbon source has previously been shown to be permissive for
growth of various yeast MDH mutants (1–3). MDH3 levels in
whole cell extracts from representative transformants were
examined by immunoblotting. As shown in Fig. 4, MDH3
polypeptides are all well expressed from the four plasmid-borne
genes (lanes A–D), although levels of the wild type protein are
approximately 2-fold higher than mutant enzyme levels. Total
cellular MDH activities are also similar, with values ranging
from 0.5 to 1.0 units/mg cellular protein as determined in three
independent experiments. These levels are similar to those
obtained from a yeast strain with an intact MDH3 locus and
containing disruptions in MDH1 and MDH2 genes (3), suggest-
ning that the plasmid-encoded wild-type and mutant forms of
MDH3 are stably expressed and fully active. The size differ-
ences among the MDH3 polypeptides are also apparent. As
shown in Fig. 4, polypeptides containing the 12-residue MDH2
amino-terminal extension (lanes C and D) migrate more slowly
during SDS-polyacrylamide gel electrophoresis than those with
authentic MDH3 amino termini (lanes A and B). The polypep-
tides containing carboxyl-terminal SKL deletions (lanes B and
D) also migrate slightly more rapidly than their counterparts
retaining the MDH3 carboxyl terminus (lanes A and C). The
latter difference is more apparent in extracts from yeast trans-
formants containing an MDH3 gene disruption but with an intact
MDH1 locus (lanes A’ and B’).

Cellular Localization and Activity of Mutant Forms of
MDH3—To examine cellular localization of mutant forms of
MDH3, yeast transformants containing all three MDH genomic
disruptions and thus expressing only the plasmid-borne
MDH3 genes (cf. Fig. 4, lanes A–D) were used for cellular
fractionation. Organellar pellets and soluble cytosolic fractions
were used for enzyme assays and immunoblot analysis. As mentioned Procedures." Fractions collected from the gradients Nycodenz gradient centrifugation as described under "Experimental Procedures." were prepared, and the organellar pellets were resolved using Nycodenz gradient centrifugation of organellar pellets obtained from a yeast strain containing chromosomal disruptions of MDH1, MDH2, and MDH3 and transformed with centromeric plasmids pRSMDH3 (A), pRSMDH3ΔSKL (B), pRSMDH2/3 (C), or pRSMDH2/3ΔSKL (D) and from a yeast strain containing chromosomal disruptions of MDH2 and MDH3 and transformed with pRSMDH3 (A') or pRSMDH3ΔSKL (B'). Amino- and carboxyl-terminal sequence differences among the forms of MDH3 are illustrated at the bottom of the figure, with letters corresponding to lane designations.

Fig. 3. Aligned terminal sequences from yeast MDH isozymes. Amino acid sequences from amino and carboxyl-terminal regions of MDH1 (1), MDH2 (2), and MDH3 (3) are aligned to show identical (lines) and similar (dots) residues. The mitochondrial targeting presequence of MDH1 is shown in brackets. Total residue numbers for the mature polypeptides are indicated in parentheses.

![Alignment of terminal sequences from yeast MDH isozymes](http://example.com/alignment.png)

Fig. 4. Expression of mutant forms of MDH3. Immunoblot analysis using antiserum against MDH3 and MDH1 was conducted with whole cell extracts (100 μg) obtained from a yeast strain containing disruptions of MDH1, MDH2, and MDH3 and transformed with centromeric plasmids pRSMDH3 (A), pRSMDH3ΔSKL (B), pRSMDH2/3 (C), or pRSMDH2/3ΔSKL (D) and from a yeast strain containing chromosomal disruptions of MDH2 and MDH3 and transformed with pRSMDH3 (A') or pRSMDH3ΔSKL (B'). Amino- and carboxyl-terminal sequence differences among the forms of MDH3 are illustrated at the bottom of the figure, with letters corresponding to lane designations.

![Immunoblot analysis](http://example.com/immunoblot.png)

Glucose Inactivation Studies of Cytosolic Forms of MDH3—To determine whether cytosolic forms of MDH3 are subject to glucose-induced degradation, the pRS plasmids encoding authentic MDH3 as a control and the MDH3ΔSKL and MDH2/3ΔSKL mutant enzymes were transformed into a yeast strain containing a single disruption of the MDH3 gene. The transformants were grown with acetate as a carbon source and then transferred to glucose medium as described above. Cellular levels of MDH3 and MDH1 were monitored by immunoblot analysis as shown in Fig. 6, changes in levels of the cytosolic forms of MDH3 (MDH3ΔSKL (B) and MDH2/3ΔSKL (D)) closely paralleled those of authentic MDH3 (A) and of MDH1 (A, B, and D), i.e. a gradual diminution due to glucose repression of expression. Neither cytosolic form demonstrated the pattern of rapid degradation associated with MDH2 (cf. Fig. 2). Thus, cytosolic localization alone is insufficient for glucose-induced degradation of MDH3. Also, although the amino-terminal ex-
tension of MDH2 was previously shown to be essential for glucose-induced degradation of MDH2 (13), the addition of this extension to the amino terminus of a cytosolic form of MDH3 does not render this enzyme susceptible to rapid degradation.

Metabolic Function of Cytosolic Forms of MDH3—Mislocalization of catalytically active forms of yeast MDH3 to the cytosol and the availability of a mutant with a disruption of the MDH2 genomic locus affords a direct test for interchangeable functions of the malate dehydrogenase isozymes in specific metabolic pathways. The pRS plasmids carrying wild type and mutant MDH3 genes were therefore transformed into a yeast strain containing a single chromosomal disruption of the MDH2 gene. Resulting Ura" transformants were then tested for the ability to utilize acetate or ethanol as a carbon source. The MDH2 disruption strain is able to grow on rich medium but unable to grow on minimal medium with either of these carbon sources (2). As illustrated in Fig. 7, transformation of this strain with plasmids encoding MDH3 (A) or MDH2/3 (C), the peroxisomal forms of this isozyme, does not alter the auxotrophic growth phenotype on ethanol plates. Transformation with plasmids encoding the cytosolic forms of MDH3 (MDH3ΔSKL (B) and MDH2/3ΔSKL (D)), in contrast, restores the ability of the MDH2 disruption strain to grow on minimal medium with ethanol. Growth rate studies with liquid cultures were also conducted; these confirmed that doubling times in minimal medium with ethanol as the carbon source are similar for transformants containing the cytosolic forms of MDH3 (8 and 8.5 h) and for the parental yeast strain (8 h), whereas no growth was detected after a 48-h period for transformants containing the peroxisomal forms of MDH3. From previous (3) and current estimates using extracts from various disruption strains grown on ethanol or acetate, the cellular malate dehydrogenase activity attributable to MDH2 is normally 3-5-fold greater than that attributable to MDH3. Thus, despite lower specific activities for the cytosolic forms of MDH3 as compared with that for authentic MDH2, the former can apparently function to correct metabolic lesions associated with loss of MDH2.

DISCUSSION

Two types of peroxisomal targeting signals, termed PTS1 and PTS2, have been identified and are described in recent reviews of organellar import and biogenesis (7, 8). The SKL carboxyl-terminal tripeptide of the MDH3 isozyme of malate dehydrogenase in S. cerevisiae is a canonical type 1 PTS. In this report, we demonstrate that MDH3 is localized to microbody organelles isolated by density gradient centrifugation and that removal of the SKL tripeptide results in mislocalization of the enzyme to the soluble cytosolic fraction of cellular lysates. Interestingly, the plant homologue of yeast MDH3 contains a type 2 PTS, in this case an amino-terminal extension of 37 residues, which is proteolytically removed upon organellar import (31). The plant malate dehydrogenase extension has also been demonstrated to be essential for import (32). Distinct import mechanisms for proteins containing the two types of PTS motifs have been implicated by analyses of mutants with defects in peroxisome assembly (7, 8). Thus, although the yeast and plant enzymes are structurally similar (43% residue identity; Ref. 3) and presumably functionally equivalent, it is likely that relatively recent evolutionary events involved acquisition of different import motifs.

The metabolic function of yeast and plant peroxisomal malate dehydrogenases is believed to be catalysis of a reaction in the glyoxylate cycle. This cycle is important during plant seedling germination for conversion of storage fatty acids to gluconeogenic precursors and in yeast allows growth with fatty acids or C2 carbon sources such as acetate and ethanol. The report by Singh et al. (16) that disruption of the S. cerevisiae MDH3 gene results in an inability to grow with oleate as a carbon source supports a role in glyoxylate metabolism. However, we have been unable to duplicate this observation with our disruption mutants, suggesting there may be some strain-specific differences in this pathway.

The cytosolic form of MDH3 obtained by removal of the carboxyl-terminal SKL appears to retain full catalytic activity. This is not surprising given sequence homology with mitochondrial MDH1 and cytosolic MDH2, which lack this carboxyl terminus. Also, recent reports (33, 34) suggest that proteins targeted to the peroxisomal matrix may attain a folded state and form oligomeric complexes prior to peroxisomal import. Thus, cytosolic formation of an active MDH3 dimer may be one step in the normal import pathway. Because the cytosolic form of MDH3 is catalytically active and stably expressed, we have been able to test the competence of this isozyme for replacement of MDH2 in yeast strains containing an MDH2 gene disruption. Cytosolic MDH3 was found to restore the ability of these strains to grow on C2 carbon sources. Thus, despite some significant differences in physical properties of the two isozymes, e.g. respective pl values of 8.6 and 4.4 for MDH3 and MDH2 (3), function in gluconeogenesis is apparently not dependent on unique structural attributes of MDH2. However, cellular growth rate studies are a relatively gross measure of function, and conclusions about equivalence of metabolic contributions will require finer methods for measuring function in vivo as well as reciprocal compartmental exchanges of MDH isozymes. In related previous experiments, we attempted to obtain cytosolic localization of mitochondrial MDH1 by removal of the 17-residue amino-terminal targeting presequence. However, we found that this did not prevent mitochondrial import.
in vivo (6). We are currently altering possible cryptic internal targeting signals with the intent of testing metabolic functions of cytosolic MDH1 for comparison with our results for MDH3.

We have found that, unlike gluconeogenic enzymes including MDH2 (13) and other glyoxylate pathway enzymes including malate synthase (11) and isocitrate lyase (12), MDH3 is not subject to rapid glucose-induced degradation. Cytosolic localization of MDH3 following removal of the SKL tripeptide does not increase susceptibility to the phenomenon. This result was not unexpected since we previously found that removal of the amino-terminal 12-residue extension of MDH2 produces an active enzyme refractory to glucose-induced inactivation and degradation. Thus, catalytic activity and cytosolic localization are insufficient attributes to target malate dehydrogenase for degradation. Also, addition of the 12-residue amino-terminal extension of MDH2 to peroxisomal or cytosolic forms of MDH3 has no effect on turnover, suggesting that, while this extension is essential for rapid turnover of MDH2 (13), there are other unique structural features of MDH2 that are recognized by components of the inactivation/turernover pathway. A final conclusion from current and previous results is that the amino-terminal extension of MDH2 is not critical for cellular location since its removal has no effect on cytosolic localization of MDH2 and its addition does not hinder peroxisomal localization of authentic MDH3. In future studies, we plan to extend these studies to correlate unique structural features of the malate dehydrogenase isozymes with differential expression and compartmental function.

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