Assembly and Function of a Cytosolic Form of NADH-specific Isocitrate Dehydrogenase in Yeast*

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Mitochondrial NAD-dependent isocitrate dehydrogenase catalyzes a rate-limiting step in the tricarboxylic acid cycle. Yeast isocitrate dehydrogenase is an octomer composed of two subunits (IDH1 and IDH2) encoded by different genes and possessing independent mitochondrial targeting presequences. Oligonucleotide-directed mutagenesis was used to remove the presequences from each gene and from both genes carried on centromere-based expression plasmids. Effects on cellular localization were examined in a yeast strain containing chromosomal disruptions of IDH1 and IDH2 loci. Each subunit was found to be dependent upon its presequence for mitochondrial localization, and the subunits are independently imported into mitochondria under most growth conditions. Furthermore, an active holoenzyme can be assembled in the cytosol and this "cytosolic" form of isocitrate dehydrogenase can reverse the acetate-growth phenotype characteristic of the ΔIDH1/ΔIDH2 disruption strain, indicating functional replacement of the mitochondrial enzyme. However, transformants containing plasmids lacking either the IDH1 or IDH2 presequence coding regions were unexpectedly found to be capable of growth on acetate medium. Further investigation demonstrated that cellular localization of the IDH1 subunit can be biased by this stringent growth pressure.

NAD-dependent isocitrate dehydrogenase in eucaryotic organisms is a key enzyme in cellular energy metabolism. The oxidative decarboxylation of isocitrate is considered an important control point for flux through the tricarboxylic acid cycle since isocitrate dehydrogenase is a complex oligomeric enzyme subject to extensive allosteric regulation by energy charge (1). The active form of isocitrate dehydrogenase in Saccharomyces cerevisiae is an α₄β₄ octomer with subunits designated IDH1 and IDH2 (2, 3). The deduced amino acid sequences from the cloned genes and amino-terminal sequence analysis (4, 5) indicate that IDH1 and IDH2 are synthesized as precursors of 360 and 369 amino acids, respectively, and are processed upon mitochondrial import to yield mature polypeptides of 349 and 354 amino acids. IDH1 and IDH2 share 42% residue identity, and both subunits share an approximate 32% residue identity with the NADP-dependent isocitrate dehydrogenase of Escherichia coli (6). For the latter enzyme, crystallographic analyses to 2.5 Å have been completed (7, 8). Based on similarity with the procaryotic enzyme, mutagenesis of the putative isocitrate binding sites of the yeast subunits and kinetic analyses led to a model for IDH1 function as a regulatory subunit and IDH2 function in catalysis (9).

The presequences of IDH1 and IDH2 have features characteristic of many mitochondrial presequences (10, 11); each contains several positively charged and hydroxylated but no acidic amino acid residues. The presequences of many mitochondrial proteins have been shown to be essential and sufficient for import. Some exceptions include yeast mitochondrial malate dehydrogenase, which has a dispensable presequence (12), and the β subunit of yeast F₄-ATPase, which contains redundant import signals, two residing within the presequence and the third within the mature protein (13). In this report, we examine the requirement for presequences for mitochondrial localization of IDH1 and IDH2 subunits and also examine the independence of import of the two subunits of this multimeric enzyme.

Compartmentalization of different metabolic pathways in eucaryotic cells frequently involves isozymes which catalyze similar reactions in different cellular compartments. The two mitochondrial and single cytosolic isozymes of isocitrate dehydrogenase in S. cerevisiae are good examples of this phenomenon. The mitochondrial tricarboxylic acid cycle enzyme is distinguished from the other two homodimeric NADP-specific isozymes (14, 15) by cofactor specificity, oligomeric composition, and allosteric sensitivity. Yeast strains containing disruptions in either or both IDH1 and IDH2 chromosomal loci share a dramatic growth phenotype, an inability to grow with acetate as a carbon source (4, 5), with some other tricarboxylic acid cycle mutants including those lacking mitochondrial malate dehydrogenase (16) or citrate synthase (17). This phenotype for strains lacking NAD-specific isocitrate dehydrogenase indicates that the residual mitochondrial NADP-specific isocitrate dehydrogenase cannot compensate for tricarboxylic acid cycle function, suggesting that NADH is the essential product of this reaction. In this report, we test whether a catalytically active NAD-specific enzyme can be assembled in the cytosol and whether reducing equivalents produced in that compartment can compensate for loss of the mitochondrial reaction.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions—Yeast strains were grown in liquid cultures or on 2% agar plates containing rich YP medium (1% yeast extract, 2% bactopeptone). All carbon sources were added to a final concentration of 2%. The parental haploid yeast strain used in these studies was S173-6B (MATa leu2-3, 112 his3-1 ura3-52 trp1-289; Ref. 18). The ΔIDH1/ΔIDH2 double disruption strain was constructed with this strain as described previously (5). The lithium acetate protocol (19) was used for yeast transformation. Transformants were selected on agar plates containing minimal YNB medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, pH 6.0) with appropriate supplements of 20 μM/ml to satisfy auxotrophic requirements for growth. Cell growth was measured spectrophotometrically at A₆₀₀ nm.

Recombinant DNA Techniques—DNA manipulation (ligation, amplification, hybridization, etc.) followed standard techniques of Sambrook et al. (20).
Both IDH1 and IDH2 genes were previously subcloned into a multiplicity clone vector (9). For the current study, to express the subunits at normal cellular levels, a 3.8-kilobase pair BamH1/HindIII fragment containing both genes was removed from the 2-μm plasmid and subcloned into pRS316, a centromere-based plasmid with a yeast URA3 gene for selection (21). To ensure a full-length promoter for the IDH1 gene, it was necessary to restore a BamH1 restriction site of IDH1 and IDH2 and were conducted simultaneously. Two 30-mer oligonucleotides complementary to regions flanking the sequence coding regions (–ATGGGACGAGAATAAGGCTGCTGTC for IDH1 and –AATATTTTTAATATGTCAGTGAACG for IDH2) were synthesized for loopout mutagenesis and a 28-mer oligonucleotide (–AGGGCGGGATCCGACCGCGGTGG) to change a SacI restriction site in the multicloning region to an SphI site was synthesized for use as a selective primer. All four mutagenic primers were used in a single synthesis reaction with single stranded template plasmid DNA prepared from the pRS316 plasmid carrying IDH1 and IDH2 genes. The ratio of loopout primers, BamH1 primer, and selective primer was 10:2:1. After strand synthesis and transformation of mutS E. coli, the plasmid population was subjected to two rounds of amplification and digestion with SacI to eliminate the template plasmid. The final plasmid population was transformed into E. coli strain DH5αF′ (22). Colony hybridization was used to identify three types of mutant plasmids, all bearing the BamH1 restriction site, plus (a) both wild type IDH1 and IDH2 genes, (b) the presequence deleted form of IDH1 with the wild type IDH2 gene, or (c) the presequence deleted form of IDH2 with the wild type IDH1 gene. All mutations were confirmed by nucleotide sequence analysis.

A 2.5-kilobase BglII restriction fragment containing the IDH1 promoter region was cloned from the original genomic isolect (4) into the BamH1 site upstream of either the wild type or the presequence deleted form of IDH1, generating plasmids, respectively, designated pDHU1/IDH2 and p3α1DHU1/IDH2. The correct orientation of the BglII promoter fragment was confirmed by nucleotide sequence analysis. XbaI restriction fragments from the two plasmids carrying the promoter and IDH1 coding regions were used to replace XbaI fragments in a plasmid bearing the presequence deleted form of IDH2, generating plasmids, respectively, designated pDHU1/α1IDH2 and p3α1DHU1/α1IDH2.

Cellular Fractionation and Activity Assays—Yeast whole cell protein extracts were prepared using glass bead lysis as described previously (16). Cell fractionation to produce mitochondrial and cytosolic fractions was performed as described by Daum et al. (23). For enzyme assays and immunoblot analyses, mitochondrial pellets were lysed in a buffer containing 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 0.5% Triton X-100. 5 mM ATP or ADP was added as indicated. Direct amino-terminal sequence analysis of the mature enzymes was performed as described previously (3); units are expressed as micromoles of isocitrate-dependent NAD⁺ reduction per min/mg of protein. Protein concentrations were determined by the Bradford dye-binding method (24).

For oxygen consumption experiments, mitochondrial pellets were resuspended to 10 mg/ml in a buffer containing 65 mM mannitol, 10 mM KPO₄ (pH 6.5), 10 mM Tris maleate (pH 6.5), 10 mM KCl, and 0.1 mM EDTA. Rates of oxygen consumption were measured in a Clarke-type polarographic oxygen electrode as described by Ohnishi et al. (25). State III respiratory rates were measured in the presence of 334 μM ADP with 13.3 mM succinate, 10 mM Tris-maleate (pH 7.4), 1 mM EDTA, and 0.5% Triton X-100. All respiratory substrates and ADP solutions were adjusted to pH 6.5.

Protein Gel Analyses—Samples of whole cell or subcellular protein extracts were electrophoresed on 10% polyacrylamide-SDS gels and electrophoresed to polyvinylidene difluoride filters. The filters were incubated as described previously using a 1:500 dilution of anti-yeast IDH1 antiserum (3), anti-yeast IDH2 antiserum (16), or anti-yeast MDH1 antiserum, or anti-yeast FBA1 antiserum (10, 11). To determine requirements for mitochondrial localization of the subunits, deletions of the presequence coding regions were constructed by oligonucleotide-directed mutagenesis as described under “Experimental Procedures.” These deletions preserve the initiator methionine codons and the coding regions for the mature polypeptides. A complete set of four plasmids was constructed by subcloning. These include a plasmid carrying both wild type genes (pDHU1/IDH2), a plasmid carrying both presequence deletions (p3α1DHU1/α1IDH2), and plasmids lacking either the IDH1 or IDH2 presequence (p3α1DHU1/IDH2 and pDHU1/α1IDH2, respectively).

The pRS plasmids were transformed into a haploid yeast strain (ΔIDH1/ΔIDH2) containing a deletion/LEU2 insertion disruption of the IDH1 chromosomal locus and a deletion/HIS3 insertion disruption of the IDH2 chromosomal locus (5). Resulting Ura+ transformants were isolated and cultivated in rich medium with glycerol plus lactate or ethanol, carbon sources permissive for growth of the ΔIDH1/ΔIDH2 disruption mutant. Immunoblot analysis of whole cell protein extracts was conducted to examine plasmid-borne expression of IDH1 and IDH2. As shown in Fig. 1, IDH1 and IDH2 levels are comparable with each other under both growth conditions in extracts from transformants expressing both wild type genes (pDHU1/IDH2, lanes 1) or the IDH2 presequence deletion gene (pDHU1/α1IDH2, lanes 3). This is also true for transformants expressing both presequence deleted genes (p3α1DHU1/α1IDH2, lanes 4) but overall cellular levels of both subunits are approximately 50% lower than wild type. In contrast, levels of IDH1 are reduced relative to IDH2 in transformants expressing the IDH1 presequence deletion gene (p3α1DHU1/IDH2, lanes 2); in fact, long exposure times are necessary to visualize IDH1 in extracts from the ethanol-grown transformant. No larger forms of IDH1 or IDH2 are immunologically detectable in any of the samples.
samples, indicating no accumulation of precursor forms of either subunit.

Enzyme assays of the whole cell extracts (Table I, A) show measurable IDH activity in the pDH1/IDH2 transformant (the levels are approximately 2-fold higher than those measured for the parental strain) and in the pΔIDH1/ΔIDH2 transformant with both carbon sources. No activity was measurable for the pΔIDH1/ΔIDH2 transformant despite the presence of both subunits, suggesting that the subunits may be localized in different cellular compartments preventing formation of the holoenzyme in vivo. This result, along with mixing experiments performed with extracts from ΔIDH1 and ΔIDH2 disruption mutants, also indicates that assembly of holoenzyme does not occur in vitro under these conditions. Traces of activity with glycerol plus lactate and with ethanol were detected in the pΔIDH1/IDH2 transformant, perhaps due to differential localization or simply to low cellular levels of IDH1 (cf. Fig. 1, lanes 2).

In another sensitive test for formation of holoenzyme, the whole cell extracts from glycerol/lactate-grown transformants were electrophoresed on a non-denaturing polyacrylamide gel and the gel treated with an IDH activity stain as described under "Experimental Procedures" (Fig. 2A). With this method, a trace of IDH activity, relative to strong staining for the extracts from pDH1/IDH2 and pΔIDH1/ΔIDH2 transformants (lanes 1 and 4, respectively), is detected in the extract from the pΔIDH1/IDH2 transformant (lane 2). Again, no activity is observed for the pDH1/ΔIDH2 transformant (lane 3). Importantly, the activity stains indicate similar electrophoretic mobilities suggesting formation of native, presumably octomer, holoenzyme in three types of transformants.

Similar patterns of relative expression were obtained for transformants grown on glucose (data not shown), which is also a permissive carbon source; however, overall immunchemical levels and activities were 3–5-fold lower due to glucose repression of IDH1 subunit levels (27).

Cellular Localization of IDH1 and IDH2 in Transformants—To determine the effects of presequence deletions on localization, cellular fractionation was conducted as described under "Experimental Procedures" to produce cytosolic and mitochondrial fractions from the four types of transformants cultured in rich medium with glycerol plus lactate as the carbon source. Immunoblot analysis (Fig. 3) demonstrates predominant mitochondrial localization of IDH1 and IDH2 in the transformant carrying pDH1/IDH2 (lanes 1). In the pΔIDH1/IDH2 transformant (lanes 2), normal levels of the processed IDH2 subunit are detected in mitochondria whereas IDH1 is undetectable in either fraction. This is generally consistent with immunoblot analysis of whole cell extracts (cf. Fig. 1) and suggests that mislocalization of IDH1 to the cytosol may result in instability and degradation. In the pΔIDH1/ΔIDH2 transformant (lanes 3), IDH2 is present in the cytosolic fraction and appears to be stably expressed in that compartment; the IDH1 subunit is mitochondrial, confirming that the absence of IDH activity in this transformant is due to differential compartmentation of the subunits. In the transformant containing pΔIDH1/ΔIDH2 (lanes 4), IDH1 and IDH2 subunits are both found in the cytosolic fraction. The efficiency of cellular fractionation in these experiments is demonstrated by immunoblot analysis of mitochondrial malate dehydrogenase (MDH1), and of cytosolic malate dehydrogenase (MDH2) as described under "Experimental Procedures." Reduced mitochondrial levels of IDH1 in lane 2, M, relative to whole cell levels (Fig. 1, lanes 2) are attributed to instability of the subunit during the period of fractionation.

Formation of an Active IDH Enzyme in the Cytosol—Since deletion of both presequences results in cytosolic localization of IDH1 and IDH2, the ability of the subunits to assemble to form an active enzyme in this compartment was examined by measuring IDH activity in the cellular fractions (Table II, A). In both the parental strain and in the transformant carrying pDH1/IDH2, IDH activity is exclusively mitochondrial. In these strains, mitochondrial specific activities exceed cellular specific activities by 20-fold with growth on glycerol/lactate and by approximately 3-fold with growth on ethanol. The trace of IDH activity detected in the pΔIDH1/ΔIDH2 transformant also appears to be associated with the mitochondrial fraction, despite our inability to detect IDH1 in that fraction with immunoblots. No measurable activity was detected in transformants contain-

**Table I**

| IDH expressed | A | B |
|--------------|---|---|
|              | Glycerol/lactate | Ethanol | Acetate |
| pDH1/IDH2    | 0.07 | 0.14 | 0.26 |
| pΔIDH1/IDH2  | Trace | 0.01 | 0.02 |
| pDH1/ΔIDH2   | 0.05 | 0.02 | 0.09 |
| pΔIDH1/ΔIDH2 | 0.05 | 0.02 | 0.07 |

**Fig. 2.** Electrophoretic analysis of IDH in yeast transformants. Whole cell protein extracts (100 μg) prepared from the parental yeast strain (P) or from a yeast ΔIDH1/ΔIDH2 disruption strain transformed with pDH1/IDH2 (lanes 1), pΔIDH1/IDH2 (lanes 2), pDH1/ΔIDH2 (lanes 3), or pΔIDH1/ΔIDH2 (lanes 4) were electrophoresed on non-denaturing polyacrylamide gels and stained for enzyme activity as described under "Experimental Procedures." Transformants were cultivated in YP medium with the indicated carbon sources.
Yeast Isocitrate Dehydrogenase

Yeast isocitrate dehydrogenase (IDH) is a key enzyme in the tricarboxylic acid (TCA) cycle. Its activity is essential for the metabolism of carbohydrates and is critical for the growth of yeast on acetate as a carbon source.

### Table II

Relative compartmental specific activity of isocitrate dehydrogenase in yeast transformants

| Compartment | IDH expressed | A | B | C | D |
|-------------|---------------|---|---|---|---|
| Mitochondria | pIDH1/ΔIDH2 | 22 (20)* | 2.9 (3.4) | 6.8 (4.7) | 3.1 |
| pΔIDH1/ΔIDH2 | 15 | 2.5 | 0.3 | 0.3 |
| pIDH1/ΔIDH2 | 0 | 0 | 0 | 0 |
| pIDH1αIDH2 | 0.5 | 0.5 | 0.6 |
| Cytosol | pIDH1/ΔIDH2 | 0 (0) | 0.1 (0) | 0.2 (0) |
| pΔIDH1/ΔIDH2 | 0 | 0 | 0.4 |
| pIDH1/ΔIDH2 | 0 | 0 | 0.8 |
| pIDH1αIDH2 | 0.5 | 0.5 | 0.6 |

* Values for the parental strain are indicated in parentheses.

### Table III

Rates of oxygen consumption of isolated mitochondria

| IDH expressed | Relative rate of mitochondrial oxygen consumption |
|---------------|--------------------------------------------------|
| IDH1/ΔIDH2 | 0.8 |
| ΔIDH1/ΔIDH2 | 0.5 |
| pIDH1/ΔIDH2 | 1.0 |
| pΔIDH1/ΔIDH2 | 0.9 (0.4) |
| pIDH1αIDH2 | 0.5 |
| pIDH1αΔIDH2 | 1.2 |

* Values in parentheses were measured for mitochondria from transformants grown with acetate as the carbon source.

To better understand the phenotypic changes conferred by IDH expression, liquid culture growth rates were measured. As shown in Table IV, all four transformant strains as well as the disruption strain grew well with ethanol as a permissive carbon source, with doubling times ranging from 5.3 to 6.4 h. With acetate, which is non-permissive for the disruption strain, culture doubling times for the transformants ranged from 6.0 to 12.0 h. Based on these growth rate values, the cytosolic form of IDH does permit growth of the pΔIDH1/ΔIDH2 transformant on acetate but does not completely restore parental rates of growth. Growth of the pΔIDH1/ΔIDH2 and pIDH1/ΔIDH2 transformants on acetate suggested formation of active enzyme in these strains and this was confirmed by enzyme assays (Table I, B) and electrophoretic staining (Fig. 2B) conducted with the whole cell extracts.

To determine the basis for growth on acetate, cell fractionation was conducted to define the cellular localization of IDH subunits and activity in acetate-grown transformants. Immunological analysis (Fig. 4) demonstrates that for acetate-grown transformants (lanes 1) or pIDH1/ΔIDH2 (lanes 4), localization of both subunits is similar to that previously observed with glycerol/lactate or ethanol, i.e. mitochondrial in the former and cytosolic in the latter. The relative specific activities for cellular fractions from these transformants (Table II, B) demonstrate compartmental distribution of activity compatible with co-localization of the subunits. In the acetate-grown pΔIDH1/ΔIDH2 transformant, IDH2 is primarily mitochondrial (lanes 2) as previously observed; however, significant levels of IDH1 are now detectable in the mitochondrial fraction. A significant enrichment in mitochondrial specific activity is also apparent (Table II, B), suggesting that formation of a mitochondrial holoenzyme accounts for growth of this transformant on acetate. In the acetate-grown IDH1/ΔIDH2 transformant, the presequence-deleted form of IDH2 is primarily cytosolic (lanes 3), as previously observed with glycerol/lactate; however, IDH1 levels in the mitochondrial and cytosolic fractions now appear to be equivalent. Co-localization of IDH1 and IDH2 in the cytosol and the enrichment in IDH specific activity in that compartment (Table II) suggest that it is formation of a holoenzyme in the cytosol which supports growth of this transformant on acetate. Mitochondria isolated from acetate-grown transformants containing pIDH1/ΔIDH2 or pΔIDH1/ΔIDH2 were also found to be unable to utilize citrate as a respiratory substrate (values in parentheses in Table III), supporting of primary cytosolic localization of the holoenzyme in these strains.
TABLE IV
Growth rates of IDH transformants
Yeast strains were cultivated on YP medium with carbon sources added to 2%. Growth rates were measured spectrophotometrically as described under "Experimental Procedures" and are expressed as culture doubling times.

| IDH expressed | Doubling time (h) | Ethanol | Acetate |
|---------------|------------------|---------|---------|
| ΔIDH1/ΔIDH2   | 6.3              | NG*     |         |
| pΔIDH1/IDH2   | 5.3              | 6.0     |         |
| pΔIDH1/ΔIDH2  | 5.6              | 8.0     |         |
| pΔIDH2/ΔIDH2  | 6.1              | 12.0    |         |
| pΔIDH1/ΔIDH2  | 5.3              | 9.4     |         |

* NG, no growth, indicates the inability to double during a 24-h period.

Fig. 4. Localization of IDH1 and IDH2 in yeast transformants cultivated with acetate. Cytosolic (C, 50 μg) and mitochondrial fractions (M, 50 μg) prepared from a yeast ΔIDH1/ΔIDH2 disruption strain transformed with pΔIDH1/IDH2 (lanes 1), pΔIDH1/ΔIDH2 (lanes 2), pΔIDH2/ΔIDH2 (lanes 3), or pΔIDH1/ΔIDH2 (lanes 4) were used for immunoblot analysis of IDH1, MDH1, and MDH2 as described under "Experimental Procedures."

DISCUSSION

In this study we have examined the structural requirements for localization and assembly of the subunits of a multimeric mitochondrial enzyme. The factors which determine localization of the IDH1 and IDH2 subunits of yeast mitochondrial NAD-dependent isocitrate dehydrogenase appear to be quite different. For IDH2, irrespective of growth conditions, the presence or absence of its mitochondrial targeting presequence appears to be the single determinant for respective mitochondrial or cytosolic localization. The subunit is stably expressed to similar cellular levels in either compartment. For IDH1, localization and stability are affected by the presence or absence of its presequence; however, other important factors appear to be growth conditions and the compartmental location of IDH2. In acetate-grown cells, for example, IDH1 is mitochondrial even in the absence of its presequence (the pΔIDH1/ΔIDH2 transformant) whereas cytosolic levels of IDH1 are significant even in the presence of its presequence when IDH2 is cytosolic (the pΔIDH2/ΔIDH2 transformant). These observations suggest that holoenzyme assembly and selective pressure for NAD-isocitrate dehydrogenase activity may override IDH1 localization signals.

In terms of mechanisms for aberrant IDH1 localization in acetate-grown cells, it is important to note that in the pΔIDH1/ΔIDH2 transformant, the sizes of cytosolic and mitochondrial forms of IDH1 are similar (cf. Fig. 4, lanes 3). Since the precursor and mature forms of IDH1 are easily distinguishable electrophoretically,1 it appears that the cytosolic form has been processed. One possible explanation is suggested by a recent report describing partitioning of yeast fumarase into two cellular compartments (28); the cytosolic form results from partial insertion of the precursor form into the mitochondrion, normal removal of the amino-terminal presequence, and release from the membrane as a folded cytosolic polypeptide. If this is also the case for IDH1 in the pΔIDH1/ΔIDH2 transformant, cytosolic retention may be facilitated by the presence of and interaction with IDH2 in that compartment. Mitochondrial accumulation of IDH1 in the pΔIDH1/ΔIDH2 transformant with acetate growth would suggest that either the presequence of IDH1 is not essential for localization or that some interaction with the presequence of IDH2 may promote import. That these aberrant patterns of localization of IDH1 are observed only with growth on acetate may be due to some shift in equilibrium to favor formation of the holoenzyme which in turn seems to stabilize IDH1. In support of this notion, we have found that the presence of cytosolic IDH2 is essential for detection of cytosolic IDH1 (data not shown).

The reason for differences in stability of cytosolic forms of IDH1 and IDH2 subunits is not clear. The mature polypeptides are similar in sequence and have identical amino-terminal residues in positions one and two, so the same N-end rules for cytosolic turnover (29) should apply to both. Our results suggest that IDH2 may be the nucleus for formation of holoenzyme. If so, it might be postulated that this subunit can form a homo-oligomeric species which might afford greater stability than a monomeric form. However, a previous study indicated that IDH2 isolated from a ΔIDH1 disruption mutant behaves as a monomer in gel filtration studies (5). Also, with the yeast two-hybrid system, we find no evidence for IDH2/IDH2 or for IDH1/IDH1 interactions under conditions where IDH1/IDH2 interactions are very strong.2

Deletion of both IDH1 and IDH2 presequences results in cytosolic localization of both subunits and formation of active holoenzyme. Thus, the mitochondrial environment and/or specific mitochondrial factors are not essential for correct polypeptide folding and subunit assembly. While we cannot rule out some accumulation of mitochondrial enzyme, immunoblot analysis and oxygen consumption assays suggest such levels would be very low. Restoration of the ability of a ΔIDH1/ΔIDH2 disruption mutant to grow on acetate suggests that the "cytosolic" form of IDH can supplement metabolic requirements for tricarboxylic acid cycle function. Similar results were recently found for a genetically engineered cytosolic form of MDH1 which also complements the acetate phenotype of a ΔMDH1 disruption strain.3 An energetic requirement in both cases is NADH. In yeast, reducing equivalents from cytosolic NADH may be supplied to the respiratory chain via an externally directed mitochondrial NADH dehydrogenase (25), perhaps explaining compensatory function by the cytosolic enzymes. In any event, stoichiometric amounts of mitochondrial IDH are clearly not essential for tricarboxylic cycle function. Among these lines, a very interesting recent report by Elzinga et al. (30) presents evidence that yeast NAD-IDH can specifically bind to sites within the 5'-untranslated leaders of several mitochondrial mRNAs. Among possible functions served by this binding are regulation of mRNA stability and/or control of translation. Our results suggest that either this binding requires very low levels of IDH or that loss of the function served by mRNA binding does not dramatically reduce cellular growth with acetate.

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