A Single Gene Produces Mitochondrial, Cytoplasmic, and Peroxisomal NADP-dependent Isocitrate Dehydrogenase in *Aspergillus nidulans*

Edyta Szewczyk, Alex Andrianopoulos, Meryl A. Davis, and Michael J. Hynes

From the Department of Genetics, University of Melbourne, Parkville, Victoria 3010, Australia

Received for publication, June 19, 2001, and in revised form, July 17, 2001
Published, JBC Papers in Press, August 1, 2001, DOI 10.1074/jbc.M105645200

NADP-dependent isocitrate dehydrogenase enzymes catalyze the decarboxylation of isocitrate to 2-oxoglutarate accompanied by the production of NADPH. In mammals two different genes encode mitochondrial and cytoplasmic/peroxisomal located enzymes, whereas in *Saccharomyces cerevisiae* three separate genes specify compartment specific enzymes. We have identified a single gene, *idpA*, in the filamentous fungus *Aspergillus nidulans* that specifies a protein with a high degree of identity to mammalian and *S. cerevisiae* enzymes. Northern blot analysis and reverse transcription-PCR analysis revealed the presence of two *idpA* transcripts and two transcription start points were identified by sequencing cDNA clones and by 5’-rapid amplification of cDNA ends. The shorter transcript was found to be inducible by acetate and by fatty acids while the longer transcript was present in higher amounts during growth in glucose containing media. The longer transcript is predicted to encode a polypeptide containing an N-terminal mitochondrial targeting sequence as well as a C-terminal tripeptide (ARL) as a potential peroxisomal targeting signal. The shorter transcript is predicted to encode a polypeptide lacking the mitochondrial targeting signal but retaining the C-terminal sequence. Immunoblotting using antibody against *S. cerevisiae* Idp1p detected two polypeptides consistent with these predictions. The functions of the predicted targeting sequences were confirmed by microscopic analysis of transformants containing fluorescent protein fusion constructs. Using anti-Idp1p antibodies, protein localization to mitochondria and peroxisomes was observed during growth on glucose whereas cytoplasmic and peroxisomal localization was found upon acetate or fatty acid induction. Therefore, we have established that by the use of two transcription start points a single gene is sufficient to specify localization of NADP-dependent isocitrate dehydrogenase to three different cellular compartments in *A. nidulans*.

Isocitrate dehydrogenase catalyzes the oxidative decarboxylation of isocitrate to 2-oxoglutarate, coupled to the reduction of a dinucleotide co-factor and production of NAD(P)H. This reaction is an essential and rate-limiting step in the tricarboxylic acid cycle and 2-oxoglutarate produced by this activity can contribute to the synthesis of glutamate, which serves as a precursor for amino acids. This reaction is therefore an important branchpoint between catabolic and anabolic processes in the cell.

Although a single NADP-specific form of this enzyme is responsible for this reaction in *Escherichia coli* (1, 2), multiple isoforms of isocitrate dehydrogenase that vary in subunit structure and cofactor specificity have been reported for both lower and higher eukaryotes. In plants two different isocitrate dehydrogenase activities co-exist in the cell. NAD-dependent isocitrate dehydrogenase appears to be heteromeric as three different subunits are present in tobacco (3), and expression is coordinated with other genes of the tricarboxylic acid cycle (4). Plant NADP-dependent isocitrate dehydrogenase isoenzymes are located within the cytosol (5), mitochondria (6), and plastids (7). In mammalian cells three highly similar isoforms of NAD-dependent isocitrate dehydrogenase are localized to the mitochondrial matrix (8). The existence of at least two NADP-dependent enzymes in mammalian cells, encoded by two independent genes, has been reported. One isoenzyme is mitochondrial whereas the other is located in both the cytosol (9) and in peroxisomes (10, 11).

In *Saccharomyces cerevisiae* NAD-dependent isocitrate dehydrogenase, located in mitochondria, is a hetero-octamer composed of two similar but distinct subunits encoded by *IDH1* and *IDH2*, respectively (12–14). Gene disruption studies show that both subunits are essential for catalytic activity (15). *S. cerevisiae* contains three NADP-dependent enzymes encoded by three separate genes. Idp1p is mitochondrial and contains an N-terminal mitochondrial targeting signal (16). Idp3p is peroxisomal, lacks the mitochondrial signal (MTS) (1) but contains a C-terminal peroxisomal targeting signal (PTS1) (17, 18). Idp2p is cytoplasmic and lacks targeting signals (19). The expression pattern of the NAD-dependent enzyme is similar to other tricarboxylic acid cycle enzymes (20, 21). In contrast, mitochondrial Idp1p levels are relatively unchanged during growth on glucose or on nonfermentable carbon sources (22) suggesting that the role of Idp1p in the tricarboxylic acid cycle may be minor in comparison to NADP-dependent isocitrate dehydrogenase. Idp2p is highly regulated with mRNA and protein levels of cytosolic Idp2p elevated in cells grown on nonfermentable car-
Aspergillus Transformation—A. nidulans protoplast preparation and DNA transformation was performed using the method of Andrianopoulos and Hynes (36). Transformants from cotransformation experiments were selected using the ribAl- selectable marker plasmid pPL3 (37) on media lacking riboflavin. Southern analysis was used to confirm that transformants contained the plasmids of interest. No effects of copy number on phenotypes were observed.

Molecular Techniques—Standard methods were as described by Sambrook et al. (38). DNA for Aspergillus transformation was isolated using the High Purity Plasmid kit (Roche Molecular Biochemicals). DNA fragments were purified from agarose gels using the BresaClean DNA isolation kit (Geneworks) following the manufacturer’s specifications. DNA dephosphorylation was performed by addition of 1 unit of arctic shrimp alkaline phosphatase (U.S. Biochemical Corp.) to 50 ng of DNA in the recommended buffer and incubation for 30 min at 37 °C. Southern and Northern blotting were performed using Hybond-N+ membrane (Amersham Pharmacia Biotech) according to manufacturer’s instructions. Filters were hybridized with [α-32P]dATP-labeled probes (random primer) and processed using standard procedures (38).

For Western immunoblot analysis, total cellular extracts were prepared by grinding frozen mycelia with glass beads in extraction buffer (60 mM Tris-HCl, pH 8.0, 10% glycerol, 5 mM dithiothreitol) in the presence of protease inhibitors (2 μg/ml aprotinin, 0.5 μg/ml leupeptin, 2.5 μg/ml antipain, 1 μg/ml pepstatin A, 1 mM phenylmethylsulfonyl fluoride), and repeated freezing in liquid nitrogen followed by centrifuging to remove cellular debris. Soluble protein in cell extracts was determined by Bradford (39) method using commercially available protein assay reagent (Bio-Rad). Protein samples were electrophoresed on 8% polyacrylamide-sodium dodecyl sulfate gels as described by Sambrook et al. (38), electroblotted to nitrocellulose transfer membrane Protran (Schleicher & Schuell), and incubated with a 1:1000 dilution of rabbit anti-yeast Idp1p primary antiserum (16) and horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Promega) secondary antibody used at 1:5000. The HRP was visualized with a chemiluminescence blotting system (Roche Molecular Biochemicals) as recommended by the manufacturer.

PCR Protocols—Sequences of primers referred to in the tables are as follows: BTUB2, AGTCTTTGACCCACCGAC; BTUB3, GTCGGC GTTGGTACATGG; idpArt1, GCGTCGTTTGTGAATAAGCCC; idpArt2, CTATACCGGTTCAACCCGGC; idpGSP1, CATGATCCGCAAGG ATGTACC; idpGSP2, GACAGCCCACCTTCTCCAGC. 5′–Rapid amplification of cDNA ends (RACE) was performed using the 5′-RACE System version2 kit (Life Technologies, Inc.) with the nested gene-specific primers idpGP1 and idpGSP2, using total RNA from the wild-type strain MH9784 grown on medium containing 50 mM sodium acetate as a sole carbon source for 4 h. RT-PCR was performed as described by Ha et al. (40) using the Superscript One-Step RT-PCR system (Life Technologies, Inc.). A total of 25 PCR cycles were used for the benA control (primers BTUB2 and BTUB3), and for idpA upper (primers idpArt1 and idpGSP1) and lower (primers idpArt2 and idpGSP1).

Plasmid Construction—The 7.8-kb ApaI-PstI 1 and 3-kb EcoRV hybridizing fragments from clone 28K20 isolated from the Bacterial Artificial Chromosome (BAC) library (kindly provided by Ralph Dean, Clemson University, Clemson, SC) clone 28K20 were cloned into pBlueScript SK+ to create pES5206 and pES4758, respectively. idpA cDNA clones were isolated from a gt10 cDNA library (kindly supplied by Gregory S. May, Baylor College of Medicine, Houston, TX), made from RNA from a culture of A. nidulans grown on YPD (2% glucose) medium. The library was probed with the 2.3-kb XhoI fragment from pES4758. The cDNA inserts were subcloned into the EcoRI site of pBluescript SK+.

DNA Sequencing—Sequencing was performed by Australian Genome Research Facility (University of Queensland, Brisbane, Australia) utilizing ABI 377 automatic DNA sequencers. Samples were sequenced and labeled using the BigDye Terminator (Applied Biosystems) Chemistry followed by a single-pass gel run.

Subcellular Localization of idpA—The gpd-p::gfp:idpA construct pES4962 encoding a GFP-IdpAPTS1 (464–493) fusion protein, was generated by ligation of a 500-bp NotI-XbaI fragment containing the gpd (glycerol-3-phosphate dehydrogenase) promoter from pES4758 into the XhoI sites of pALX213. pALX213 contains the coding sequence for the enhanced variant of the green fluorescent protein (GFP) of Aquorea victoria (CLONTECH) expressed from the A. nidulans gpdA promoter (41). The NotI and HindIII sites were end-filled using the Klenow enzyme.

A. nidulans NADP-dependent Isocitrate Dehydrogenase

| Strain     | Genotype  |
|------------|-----------|
| MH1        | biAJ      |
| MH50       | ya1 su-adE20 adeE20 araA102 pyroA4 ribaB2 |
| MH10025    | MH50 transformed with pNBE8-5 and pPL3 |
| MH10029    | MH50 transformed with pALX207-10 and pPL3 |
| MH9908     | MH50 transformed with pES5020 and pPL3 |
| MH9784     | MH50 transformed with pES4962 and pPL3 |

| Table I  
| A. nidulans strains used in the present study |

| Strain     | Genotype  |
|------------|-----------|
| MH1        | biAJ      |
| MH50       | ya1 su-adE20 adeE20 araA102 pyroA4 ribaB2 |
| MH10025    | MH50 transformed with pNBE8-5 and pPL3 |
| MH10029    | MH50 transformed with pALX207-10 and pPL3 |
| MH9908     | MH50 transformed with pES5020 and pPL3 |
| MH9784     | MH50 transformed with pES4962 and pPL3 |

2 A. Andrianopoulos, unpublished data.
fragment of DNA polymerase I (Promega). The idpA::rfp construct pES5020 encoding an IdpA/MTS (1–81)–RFP fusion protein was generated by ligation of the 700-bp BamHI-NolI fragment of pBSRed1-N1 (CLONTECH) containing the coding sequence for the novel red fluorescent protein (RFP) of Discosoma sp., into the sites NcoI-NolI of pES4758 encoding an N-terminal IdpA fragment expressed from the idpA promoter. The BamHI and NcoI sites were end-filled. Riboct® cotransformants MH9784 and MH9908 that contained pES4962 or pES5020, respectively, were identified by direct fluorescence microscopy screening, and confirmed by Southern analysis.

Cells were grown for microscopic analysis on coverslips in liquid glucose minimal medium containing 10 mM ammonium tartrate for 12 h at 25 °C, and then transferred to liquid medium with the same conditions or 1% Tween 80 or 50 mM sodium acetate as sole carbon source and 10 mM ammonium chloride for another 4 h at 25 °C. Mitochondria were stained with MitoTracker Red CMXRs or Green FM (Molecular Probes) for 20 min. For each experiment, at least three cotransformants with different copy numbers were studied. For indirect immunofluorescence, cells grown on coverslips were fixed and prepared using the method described by Small et al. (42). The rabbit polyclonal antiserum against yeast Idp1p (16) was used at 1:1,000, and fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Promega) secondary antibody was used at 1:500. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) (42).

Microscopy was performed using a Reichert-Jung microscope equipped with Nomarski differential interference contrast (DIC) and fluorescence optics. For GFP and MitoTracker Green FM fluorescence and FITC immunofluorescence, the filter set used was a standard FITC set, with an excitation wavelength range of 450–495 nm, dichroic mirror at 510 nm and long pass barrier filter at 520 nm. For RFP and MitoTracker Red CMXRs fluorescence and Alexa Fluor immunofluorescence, the TRITC filter set was used with an excitation wavelength range of 546 ± 10 nm, dichroic mirror at 580 nm and long pass barrier filter at 418 nm. For cells stained with DAPI to visualize nuclei, the filter set used had an excitation wavelength range of 330–380 nm, dichroic mirror at 420 nm, and long pass barrier filter at 418 nm. Photomicrographs were captured using a Diagnostics Instruments Spot Camera and then manipulated using Photoshop 4.0 (Adobe Systems).

Nucleotide Sequence Accession Number—The sequence for the idpA gene has been deposited in GenBank® under accession no. AY040207.

RESULTS

Cloning of the idpA Gene from A. nidulans—A BLAST search performed on the University of Oklahoma A. nidulans EST data base (www.genome.ou.edu/fungal.html) revealed sequences (EST x8f0a1.f1, kb02a1.f1, and kb02a1.r1) with a high level of similarity to the NADP-dependent isocitrate dehydrogenase enzymes of S. cerevisiae (16–19), Candida tropicalis (43), and A. niger (accession no. AB000282). The EST x8f0a1.f1 clone obtained from the Fungal Genomic Stock Center (University of Kansas, Kansas City, KS) was used to probe an A. nidulans BAC library (see “Experimental Procedures”) and four hybridizing BAC clones were identified. Clones SH5, 12K11, 28H17, and 28K20 all showed an identical restriction pattern in the hybridizing region in Southern blots.

A 7.8-kb ApaI-PstI fragment was subcloned from BAC 28K20, creating pES5206. Sequencing 5.7 kb of the 5′ end of this clone revealed an open reading frame, interrupted by seven introns, with extensive similarity to Idp1p, Idp2p, and Idp3p of S. cerevisiae. The gene was designated idpA. GenBank data base searches (BLAST) of sequences upstream of the promoter region of the idpA gene also identified a portion of a second open reading frame in this clone with significant identity to the C1-tetrahydrofolate synthase from Yarrowia lipolytica (AF291429) (Fig. 1).

Analysis of idpA Gene Structure—The idpA coding region contained seven introns most of which ranged in size from 48 to 90 bp with an unusually long 299-bp first intron. Sequencing idpA cDNA clones isolated from a’ét10 library confirmed the positions of the predicted introns and revealed the presence of an additional intron (61 bp) in the 5′-untranslated region of the gene (from position −149 to −89). The independent cDNA clones also allowed the localization of start points of transcription (at −319, −279, −276, −271, and −270 relative to the first predicted ATG) and polyadenylation sites (+2249, +2250, +2252, +2291, and +2292) (Fig. 1). The position of introns, as well as their relative length, are strictly conserved between A. nidulans and A. niger.

The idpA gene is predicted to encode a 493-amino acid protein with significant similarity to all reported NADP-dependent isocitrate dehydrogenases, with a highly conserved isocitrate dehydrogenase and isopropylmalate dehydrogenase signature sequence and putative sites involved in isocitrate binding (PROSITE data base; Ref. 44). IdpA shows the highest degree of similarity to the enzyme from A. niger (85% identity and 90% similarity, with conservation across the entire protein) and the enzymes from S. cerevisiae (Idp1p, 72% identity and 82% similarity; Idp2p, 69% identity and 82% similarity; Idp3p, 66% identity and 81% similarity) (Fig. 2).

A PSORTI (psort.nibb.ac.jp) search for subcellular targeting signals in IdpA identified both an N-terminal MTS with a prescission cleavage site after amino acid 33–LRS | FS (47), as well as a C-terminal PTS1 -ARL (48, 49). These targeting signals are conserved in the A. niger enzyme. Southern blot analysis at low stringency revealed no idpA cross-hybridizing sequences in the genome of A. nidulans, indicating that idpA is unique (data not shown).

Analysis of idpA Expression—Northern blot analysis of idpA expression in mycelia grown on different carbon sources revealed the presence of two differently regulated transcripts (Fig. 3). The longer (1.9 kb) transcript correlated with the size of the cDNA clones and was readily detected during growth in the presence of glucose with lower levels present in the absence of glucose (Fig. 3B). A shorter (1.5 kb) transcript (Fig. 3A) was detected that was highly regulated by the carbon source, with induction by acetate, propionate, butyrate, and Tween 80 (a source of fatty acids, see Ref. 51).
The presence of two transcripts suggested the existence of two transcription start points in the idpA gene. The first major start point (319 bp upstream of the first translation initiation codon, Fig. 1B) determined from the idpA cDNA clones, corresponded to the longer transcript. The second major transcription start point (Fig. 1A) was identified in multiple clones generated by 5'-RACE on total RNA isolated from mycelium transferred for 4 h to 50 mM sodium acetate as the sole carbon source. Two independent clones localized the transcriptional start point to +156 and two other clones to +155 and +181, respectively. Transcription starting at this point would result in the shorter, carbon source-regulated transcript (Fig. 3A).

The expression of two transcripts was confirmed by RT-PCR. The use of primers idpArt1 specific for the longer transcript B and idpArt2 amplifying both transcripts A and B (Fig. 4A) allowed the analysis of transcription from the proposed start points. Two products of sizes consistent with the primer positions were observed. The shorter product was abundant when RNA was isolated from mycelium grown on acetate as the sole carbon source (Fig. 4B).

As the shorter transcript would lack the predicted translational start codon at +1, it is predicted that the two idpA mRNAs would result in two proteins differing at their amino terminus. Analysis of the idpA gene sequence downstream of the second transcriptional start point revealed two additional possible initiation codons (at positions +241 and +593, with reference to the first nucleotide of the idpA coding region; Fig. 1). The use of the start codon at position +241 would include the strongly conserved block of amino acids (KIKV) located between these two putative methionine residues (Fig. 2).

Western Immunoblot Analysis of IdpA—The products of the idpA gene were analyzed by means of Western blot with rabbit polyclonal antiserum against S. cerevisiae Idp1p (16). The anti-Idp1p antiserum detected two distinct protein bands of ~60 and 46 kDa in A. nidulans protein extracts (Fig. 5). The level of expression of the shorter polypeptide (Fig. 5A) was highly dependent on the carbon source with strong induction by acetate, propionate, and fatty acids. The longer polypeptide (Fig. 5B) was present at low levels in these extracts but was more readily detected in cells grown in presence of glucose in agreement with the levels of the longer transcript in the presence of glucose (Fig. 3). It is possible that extraction of this protein from mitochondria was less efficient than for extraction of the shorter cytoplasmic/peroxisomal protein (see below), thereby explaining the differences between the relative levels of the transcripts and polypeptides in the Northern (Fig. 3) and the Western (Fig. 5) analysis. The full-length IdpA protein has a predicted molecular mass of 55 kDa. Cleavage of the MTS predicts a molecular mass of 52 kDa. This may be in agreement with the size of the longer polypeptide B detected, since the A. niger purified NADP-dependent isocitrate dehydrogenase migrated more slowly than predicted (53). The size of shorter polypeptide A (46 kDa, Fig. 5) is in agreement with the pre-
form clusters randomly distributed throughout the hyphae and in the germinated spore body when transferred from 1% glucose minimal medium and grown for another 12 h in Tween 80 as a source of fatty acids (data not shown) as described previously (56). These observations indicated that both targeting signals of IdpA are fully functional.

The subcellular localization of IdpA was analyzed by immunostaining and indirect immunofluorescence with the anti-Idp1p used in the Western blot analysis. In order to co-localize IdpA within subcellular organelles, two different A. nidulans transformant strains were used. MH10025 expresses an AcuJ-GFP fusion protein possessing the MTS of carnitine acetyltransferase and lacking the C-terminal PTS1.\(^3\) MH10029 expresses an AcuE-GFP fusion targeted to peroxisomes with malate synthase PTS1.\(^2\) Immunostaining of IdpA with anti-Idp1p, and of GFP fusion proteins with anti-GFP, respectively, allowed cell compartments to be distinguished. In cells grown in 1% glucose medium, IdpA was localized in small spherical organelles and a long tubular cell compartment (Fig. 7, C and L), and was not dispersed evenly along the hyphae. Location of AcuE-GFP fusion in the same spherical organelles indicated their peroxisomal character (Fig. 7K). The AcuJ-GFP fusion localization indicated that the tubular compartment consisted of mitochondria (Fig. 7B). In the presence of acetate or Tween 80, IdpA co-localized in spherical peroxisomes with the AcuE-GFP fusion (Fig. 7, O and R and N and Q, respectively) and evenly through the cytosol. Under these conditions NADP-dependent isocitrate dehydrogenase was not detected in mitochondria (Fig. 7F, I) where AcuJ-GFP was still present (Fig. 7, E and H). The observed localization was in agreement with the IdpA protein structure and with two forms of the enzyme differentially expressed depending on the carbon source. On acetate or Tween 80, the shorter form, lacking an MTS and possessing only the C-terminal PTS1, is preferentially expressed and is localized to the peroxisomes and the cytosol. In the presence of glucose, the longer form containing an MTS is expressed and located in mitochondria. IdpA was never detected in the nucleus (data not shown) consistent with the lack of a nuclear targeting signal in the predicted amino acid sequence.

**DISCUSSION**

Previously it was shown that A. nidulans NADP-dependent isocitrate dehydrogenase has both mitochondrial and acetate inducible non-mitochondrial isozymes (31). Acetate-inducible expression of activity has been shown to be dependent on the *fauA* gene, which encodes a C6 zinc binuclear cluster protein involved in regulation of genes encoding enzymes required for acetate utilization (33, 59). Repression by glucose of acetate-inducible expression is mediated by the *creA* gene, which encodes a C2H2 zinc finger repressor (33, 36)

\(^3\) M. J. Hynes and A. Andrianopoulos, unpublished results.
We have now shown that *A. nidulans* contains a single gene encoding a highly conserved NADP-dependent isocitrate dehydrogenase. Two transcription start points result in two different protein products. The longer transcript is present at higher levels on glucose media and encodes a protein containing an N-terminal sequence capable of targeting RFP to mitochondria. The shorter transcript results in a protein that is localized to the cytoplasm and to peroxisomes. Peroxisomal targeting results from a C-terminal PTS1 (ARL), which confers localization of GFP to peroxisomes. The shorter transcript is inducible by acetate and propionate and is glucose repressible. In addition this transcript is inducible by butyrate or Tween 80 as a source of fatty acids. This pattern of regulation is similar to that for a number of genes encoding peroxisomal located proteins in *A. nidulans*: *acuD* (isocitrate lyase), *acuE* (malate synthase), and *acuJ* (carnitine acetyltransferase). Acetate induction has been found to be mediated by the *facB* gene (33), but genes involved in butyrate and fatty acid induction have not yet been identified.

The problem of targeting of NADP-dependent isocitrate dehydrogenase activity to three cellular compartments has been solved differently in different organisms. Our results show that by the use of different transcription start points two different forms of the enzyme are produced from a single gene in *A. nidulans*. A similar situation appears to be the case in *A. niger*.

In *S. cerevisiae* three separate genes encode compartment-specific enzymes (see Introduction). This is also the case for three isozymes of malate dehydrogenase in *S. cerevisiae* (20, 61, 62). In mammals two distinct NADP-dependent isocitrate dehydrogenases encoded by separate genes, as shown by phylogenetic analysis, have been found (63). The mitochondrial forms possess an N-terminal MTS, while all analyzed mammalian cytosolic enzymes contain a C-terminal tripeptide (AKL), which is a potential peroxisomal targeting sequence (PTS1) (63). The physiological importance of this has been unclear although activity has been reported in rat liver peroxisomes (64). More recently, however, Geisbrecht and Gould (10) have shown that a human gene (*PICD*) encodes activities in both peroxisomes and the cytosol and is identical to the previously cloned cytosolic gene (63). Therefore, in mammals a single gene encodes both peroxisomal and cytoplasmic forms. This situation is the same as that for the product of the shorter *A. nidulans* idpA transcript, which is also localized to both peroxisomes and cytosol. Geisbrecht and Gould (10) have suggested that the enzyme could be only poorly imported into peroxisomes so that most activity is cytoplasmic. They also suggest that the C-terminal PTS1 could be masked by oligomerization or by interaction with other proteins. It is interesting to note that we observed much higher levels of cytoplasmic IdpA under induced conditions (Fig. 7), indicating a correlation between amount of protein and cytoplasmic localization.

---

4 N. Beevers, A. Andrianopoulos, and M. J. Hynes, unpublished data.
IdpA is detected in peroxisomes and cytosol (I) or in medium containing 50 mM sodium acetate (Twe) with 1% Tween 80, or to medium lacking glucose (Gluc) minimal medium for 12 h, and then transferred to fresh media with 1% glucose (C-free) and under the control of the 10 expressing GFP fused to the N-terminal 266 amino acids of AcuJ and lacking the C-terminal PTS1 (AcuJ-GFP) (see Footnote 5) for another 4 h. Cells were fixed and immunostained using mouse anti-GFP primary antibody and Alexa Fluor-conjugated anti-mouse IgG secondary antibody to visualize AcuJ-GFP. Germlings were viewed using DIC, a TRITC filter set, and a FITC filter set. AcuJ-GFP was localized in the mitochondria (B, E, and H), and Acu-E-GFP was localized in the peroxisomes (K, N, Q) under all conditions. In glucose medium IdpA is observed in mitochondria and peroxisomes (C and L), whereas with acetate or Tween 80 IdpA is detected in peroxisomes and cytosol (F, I, O, and R).

Single genes encoding both mitochondrial and peroxisomal forms have been found for other enzymes. Peroxisomal and mitochondrial localized carnitine acetyltransferase enzymes are determined by a single gene in S. cerevisiae (65, 66). Two different transcriptional start points are used to generate two proteins. The mitochondrial form contains both an N-terminal MTS and a C-terminal PTS1, indicating that mitochondrial targeting predominates over peroxisomal targeting, whereas the peroxisomal protein lacks the MTS. Two other genes encode cytoplasmic enzymes lacking either signal (67, 68). In C. tropica, a single gene also determines two forms of carnitine acetyltransferase but in this case the peroxisomal enzyme results from alternative initiation of translation (69, 70). A similar situation for genes encoding carnitine acetyltransferase enzymes is likely in A. nidulans (57). In mammalian alanine:glyoxalate aminotransferase shows species specific localization with the proportion of enzyme targeted to mitochondria varying from 0% to greater than 90% with the remainder being peroxisomal (reviewed in Ref. 71). Similar to the situation described here for idpA, the basis for this is the use of two different transcription start points and two different initiation codons framing a mitochondrial targeting sequence. In humans and rabbits, the enzyme is only peroxisomal due to evolutionary loss of the upstream ATG leading to a protein lacking the mitochondrial targeting sequence. The use of both transcription start points in rats and marmosets results in approximately equal distribution of enzyme between mitochondria and peroxisomes. In cats exclusive use of the upstream transcription start causes most enzyme to be mitochondrial with a minor component peroxisomal, presumably due to a low frequency use of the downstream initiation codon. This again supports the functional dominance of mitochondrial over peroxisomal targeting, which may be due to a commitment to mitochondrial import occurring co-translationally before the C terminus is synthesized (71).

It is clear that NADP-dependent isocitrate dehydrogenase enzymes are highly conserved in eukaryotes and also have a conserved pattern of subcellular localization. A major role is likely to be the provision of NADPH for reducing processes in mammals (10) and in S. cerevisiae (17, 18, 30). Carbon source-dependent regulation of the cytoplasmic Idp2p is mediated by Cat8p (46, 72), whereas the peroxisomal Idp3p is regulated by fatty acid induction mediated by Oaf1p and Ppi2p (17, 18). In agreement with this, in A. nidulans cytoplasmic and peroxisomal IdpA is regulated by acetate induction mediated by the fecB gene, an apparent homologue of CAT8 (53, 59) and by fatty acid induction, suggesting a role in generating NADPH during growth on two-carbon compounds and on fatty acids as sources of carbon. An additional function for the enzyme is likely to be the generation of 2-oxoglutarate for amino acid biosynthesis. We are currently generating deletions of idpA to determine its physiological role in A. nidulans and investigating in detail the sequences involved in the regulation of this gene.

Acknowledgments—We acknowledge Lee McAlister-Henn for providing invaluable antiserum again S. cerevisiae Idp1p, Sophie Delimitrou for sequencing cDNA clones, Richard B. Todd for discussions, and Trevor Lithgow for a gift of MitoTracker dyes.

REFERENCES
1. Plaut, G. W. E. (1963) in The Enzymes (Boyer, P. D., Lardy, K., and Myerback, K., eds) Vol. 7, pp. 105–126, Academic Press, New York
2. Borthwick, A. C., Holmes, W. H., and Nimmo, H. G. (1984) Eur. J. Biochem. 141, 393–400
3. Lancien, M., Gadal, P., and Hodges, M. (1998) Plant J. 16, 325–333
4. Chen, R.-D., and Gadal, P. (1990) Plant Physiol. Biochem. 28, 141–145
5. Galvez, S., Hodges, M., Decottignies, P., Bismuth, E., Lancien, M., Songwam, R. S., Dubois, F., LeMarechal, P., Creten, C., and Gadal, P. (1996) Plant Mol. Biol. 30, 307–320
6. Galvez, S., Roche, O., Bismuth, E., Brown, S., Gadal, P., and Hodges, M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7813–7818
7. Galvez, S., Bismuth, E., Sarda, C., and Gadal, P. (1994) Plant Physiol. 103, 593–600
8. Ramachandran, N., and Colman, R. F. (1980) J. Biol. Chem. 255, 8859–8864
9. Jennings, G. T., Sechi, S., Stevenson, P. M., Tuckey, R. C., Parmelee, D., and McAlister-Henn, L. (1994) J. Biol. Chem. 269, 25128–25134
10. Geisbrecht, B. V., and Gould, S. J. (1999) J. Biol. Chem. 274, 30527–30533
11. Plaut, G. W., Cook, M., and Angachi, T. (1983) Biochim. Biophys. Acta 760, 300–308
12. Keys, D. A., and McAlister-Henn, L. (1990) J. Bacteriol. 172, 4280–4287
A Single Gene Produces Mitochondrial, Cytoplasmic, and Peroxisomal NADP-dependent Isocitrate Dehydrogenase in Aspergillus nidulans
Edyta Szewczyk, Alex Andrianopoulos, Meryl A. Davis and Michael J. Hynes

J. Biol. Chem. 2001, 276:37722-37729.
doi: 10.1074/jbc.M105645200 originally published online August 1, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M105645200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 70 references, 27 of which can be accessed free at http://www.jbc.org/content/276/40/37722.full.html#ref-list-1