Targeting of malate synthase 1 to the peroxisomes of *Saccharomyces cerevisiae* cells depends on growth on oleic acid medium

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The eukaryotic glyoxylate cycle has been previously hypothesized to occur in the peroxisomal compartment, which in the yeast *Saccharomyces cerevisiae* additionally represents the sole site for fatty acid β-oxidation. The subcellular location of the key glyoxylate-cycle enzyme malate synthase 1 (Mls1p), an SKL-terminated protein, was examined in yeast cells grown on different carbon sources. Immunoelectron microscopy in combination with cell fractionation showed that Mls1p was abundant in the peroxisomes of cells grown on oleic acid, whereas in ethanol-grown cells Mls1p was primarily cytosolic. This was reinforced using a green fluorescent protein (GFP)–Mls1p reporter, which entered peroxisomes solely in cells grown under oleic acid-medium conditions. Although growth of cells devoid of Mls1p on ethanol or acetate could be fully restored using a cytosolic Mls1p devoid of SKL, this construct could only partially alleviate the requirement for native Mls1p in cells grown on oleic acid. The combined results indicated that Mls1p remained in the cytosol of cells grown on ethanol, and that targeting of Mls1p to the peroxisomes was advantageous to cells grown on oleic acid as a sole carbon source.

**Keywords:** *Saccharomyces cerevisiae*; glyoxylate cycle; peroxisome; malate synthase 1; oleic acid.

Microorganisms are able to grow on nonfermentable carbon sources such as acetate, ethanol, or fatty acids, because they possess a glyoxylate cycle for generating four-carbon units that are suitable for biosyntheses of macromolecules. Similarly, plant seedlings can also use stored lipids as a sole carbon and energy source, by converting the acetyl-CoA product of fatty acid β-oxidation to four-carbon units using a cognate process. In those eukaryotes known to possess a glyoxylate cycle, e.g. plant seedlings and fungi, the process is thought to occur in the peroxisomal matrix.

Peroxisomes typically contain enzymes for reactions involving molecular oxygen and for metabolizing hydrogen peroxide [1]. This subcellular compartment represents the site of fatty acid β-oxidation, which in mammals is augmented by an additional process found in the mitochondria [2]. The significance of the fungal glyoxylate cycle to human health is underscored by the requirement of *Candida albicans* for the virulence factor isocitrate lyase [3]. Like the situation with *C. albicans*, *Saccharomyces cerevisiae* cells isolated from infected mammalian cells similarly up-regulate isocitrate lyase as well as malate synthase, both of which represent key enzymes unique to the glyoxylate cycle [3]. As *S. cerevisiae* is a genetically more tractable yeast than *C. albicans*, it was chosen as a model fungal system for studying the glyoxylate cycle by analysing the subcellular distribution of malate synthase 1.

The *S. cerevisiae* glyoxylate cycle (Scheme 1) consists of five enzymatic activities, some of which are represented by isoenzymes: isocitrate lyase, Icl1p [4]; malate synthase, Mls1p and Dal7p [5]; malate dehydrogenase, Mdh1p [6], Mdh2p [7] and Mdh3p [8,9]; citrate synthase, Cit1p [10], Cit2p [11,12] and Cit3p/YPR001w [13]; and aconitase, Aco1p [14] and Aco2p/YJL200c [13]. As mentioned above, isocitrate lyase and malate synthase represent key enzyme activities that are unique to the glyoxylate cycle, whereas some of the remaining enzymes, e.g. mitochondrial Cit1p, Mdh1p, and Aco1p, are shared with the citric acid cycle. Icl1p is an extraperoxisomal protein, while Mdh3p and Cit2p are peroxisomal ones. The latter two enzymes end with a C-terminal SKL tripeptide representing a peroxisomal targeting signal PTS1 [15–17].

The two malate synthases Mls1p and Dal7p are also SKL-terminating proteins that are 81% identical to one another. However, as the *MLS1* gene is highly transcribed on nonfermentable carbon sources and is essential for cell growth on these media, whereas *DAL7* is not [5], it is reasoned that only Mls1p represents the malate synthase activity specifically involved in the glyoxylate cycle. Dal7p, whose peroxisomal location remains putative, is actually thought to be involved in the metabolism of glyoxylate produced during the degradation of allantoic acid to urea [5].

Initial work on peroxisomal citrate synthase (Cit2p) led to the conclusion that the glyoxylate cycle is a peroxisomal process [12]. However, the cycle’s subcellular location is no longer clear because peroxisomal Cit2p has since been shown to be dispensable for the glyoxylate cycle [9] and, moreover, cells lacking peroxisomal malate dehydrogenase...
promoter and terminator regions of Pvu
Stockholm, Sweden) using oligonucleotide H161 (Table 1).

manufacturer's protocol (Amersham Pharmacia Biotech.,
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demonstration that Icl1p is a cytosolic enzyme [4], and that
mutants lacking functional peroxisomes grow plentifully
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malate dehydrogenase activity specifically involved in the
glyoxylate cycle is attributed to the cytosolic isoform
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for the yeast glyoxylate cycle was further reinforced by the
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ethanol or oleate.

MATERIALS AND METHODS

Strains, plasmid constructions and gene disruptions
S. cerevisiae strains, plasmids and oligonucleotides used are
listed in Table 1. Escherichia coli strain HB101 was used for
all plasmid amplifications and isolations. Construction of
strains JD1, JR85, and JR86 has been described [5]. To
remove the three codons for SKL from the MLSI gene,
single-strand mutagenesis was performed according to the
manufacturer’s protocol (Amersham Pharmacia Biotech.,
Stockholm, Sweden) using oligonucleotide H161 (Table 1).
To reintroduce the native MLSI or an MLSI variant
lacking the SKL codons back to the genomic MLSI locus,
strain JR86 was transformed with UR43-marked integrative
plasmids pB10-WT or pB10-WT ΔSKL digested with PvuII. These pUC18-based plasmids consisted of the
promoter and terminator regions of MLSI delineating the
open reading frame, with or without the codons for SKL,
and URA3 (Scheme 2). Integration of the disruption
fragments resulted in the respective strains KM10 and
KM11. Correct integration of these plasmid fragments was
verified by polymerase chain reaction using oligonucleotide
pairs H338 and H162, or H339 and H161, respectively
(Table 1, Scheme 2).

To generate null mutants devoid of Mls1p, the corre-
sponding gene was deleted by transforming strains BJ1991
[20] with an mls1Δ::LEU2 disruption fragment [5]. Cells that
had returned to leucine prototrophy were verified for
growth deficiency on ethanol and acetate media and were
designated strain KM12. The mutant phenotype was
confirmed by complementation using native MLSI carried
on a YEp352 multicopy vector, YEp352-MLS1 [5]. The
BJ1991-derived strain KM13 expressing the SKL-less
Mls1p was constructed and verified as described above for
strain KM11. YEp352-MLS1::SKL was constructed by
inserting a 2.3-kb SaI fragment containing the complete
MLS1 gene into this multicopy vector, and replacing parts
of the coding region with the single-strand mutagenized
sequence, resulting in the expression of an SKL-truncated
Mls1p (Mls1pΔSKL). The plasmid was introduced to strain
JR86, resulting in strain KM15.

To create a reporter construct based on GFP extended by the
C-terminal half of Mls1p comprising 274 amino acids of a
total of 554, PCR was applied to YEp352-MLS1 template
DNA using oligonucleotides H623 and H625 and Pfu high-
fidelity polymerase (Stratagene, La Jolla, CA, USA). The
single amplification product obtained was digested with
SphI and BglII, and ligated to an SphI- and BamHI-digested
plasmid pJR233M [21], resulting in plasmid pLW89.
Construction of the parent plasmid pJR233 is described
elsewhere [22]. Nucleic acid manipulations [23] and yeast
transformations [24] were performed as described.

Media and growth conditions
Plates contained 0.67% (w/v) yeast nitrogen base without
amino acids (Difco), 3% (w/v) agar, amino acids as
required, and either 2% (w/v) D-glucose, 2.5% (v/v) ethanol,
or 0.1 mM potassium acetate at pH 6.0. Fatty acid plates
were prepared by adjusting prior to use, plates contained
0.67% (w/v) yeast nitrogen base without
carbon source as above [25,26]. Cells were grown to late log phase.

Cells were transferred to water at a concentration of
10^7 cells mL^-1, serially diluted (1 : 10 dilutions), and culture
aliquots of 2.5 μL were applied to solid media [25,26].

Growth assays in liquid oleic acid medium were performed
following a modified protocol [25,26]. Cells were grown
overnight in synthetic medium (0.67% yeast nitrogen base
with amino acids added) containing 2% D-glucose, and the
cultures diluted to an D_600 of 0.5 in synthetic medium
containing 0.5% D-glucose and grown further with shaking
at 30 °C. Upon reaching an D_600 of 3.0 culture aliquots were
removed and diluted to an D_600 of 0.02 in synthetic media
containing 0.03 mM potassium phosphate buffer (pH 6.0),
0.1% yeast extract, and either 2% ethanol or 0.2% oleic acid
and 0.02% Tween 80 (the latter carbon source adjusted prior
Table 1. *S. cerevisiae* strains, plasmids, and oligonucleotides used. The numbers in superscript following the strains’ designation refer to their parental genotypes, e.g. JD<sup>1</sup> was derived from (1) GA1-8C.

<table>
<thead>
<tr>
<th>Strain, plasmid, or oligonucleotide</th>
<th>Description</th>
<th>Source or Reference</th>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
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<tr>
<td>(1) GA1-8C</td>
<td><em>MAT</em> _ura3-32 leu2 his3 trp1-1 ctt1-1 gal2*</td>
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<td>JD&lt;sup&gt;1&lt;/sup&gt;</td>
<td><em>dafl7Δ:HIS3</em></td>
<td>[5]</td>
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<tr>
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<td>[5]</td>
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<tr>
<td>(3) JR86&lt;sup&gt;2&lt;/sup&gt;</td>
<td><em>mls1Δ:LEU2 dafl7Δ:HIS3</em></td>
<td>[5]</td>
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<td>KM10&lt;sup&gt;3&lt;/sup&gt;</td>
<td><em>URA3</em>, expressing Mls1p&lt;sub&gt;ΔSKL&lt;/sub&gt; from the <em>MLS1</em> locus</td>
<td>This study</td>
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<td>KM1&lt;sup&gt;1&lt;/sup&gt;</td>
<td><em>URA3</em>, expressing Mls1p from the <em>MLS1</em> locus</td>
<td>This study</td>
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<tr>
<td>(4) BJ1991</td>
<td><em>MAT</em> <em>leu2</em> <em>ura3-52 trp1 pep4-3 prb1-1122 gal2</em></td>
<td>[20]</td>
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<td>This study</td>
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<td>Multicopy vector harboring native <em>MLS1</em></td>
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<td>Multicopy vector harboring a truncated <em>MLS1</em></td>
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<td>YEp352-based plasmid expressing GFP-&lt;sub&gt;ΔSKL&lt;/sub&gt;</td>
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<td>pJR233M</td>
<td>pJR233-derived vector for GFP fusions</td>
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<td>H625</td>
<td><em>5'-AGAAGCATGCGATCACAATTATGCTCAAACTCAGTGCGGCGTCGCC-3'</em></td>
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Plasma was determined at the times indicated. For vital counts, culture aliquots were removed following the specified periods and plated on solid YP medium containing 2% D-glucose for enumeration following 2 days incubation.

**Cell fractionation and immunoblotting**

Late log-phase cells were harvested by centrifugation and transferred to YP medium containing 2.5% ethanol, or 0.2% oleic acid and 0.02% Tween 80 (pH adjusted as mentioned above). Following growth for at least 9 h at 30 °C with shaking, cells were harvested by centrifugation (5000 g), and total homogenates, organelar pellets, and postorganellar supernatants were prepared as described [27]. A 10% portion of each of the fractions (postnuclear supernatant, organelar pellet or cytosolic supernatant) was used for protein precipitation. These organelar or supernatant fractions were made up to 0.5 mL with breaking buffer [27], followed by 5 μL Triton X-100 (final concentration 1% v/v) and an appropriate amount of 80% (w/v) trichloroacetic acid to obtain a 10% final concentration of trichloroacetic acid. The resulting oily pellet was washed once with a diethyl ether:ethanol mixture (1:1), which removed traces of Triton X-100 and trichloroacetic acid, and dissolved in 30 μL 0.1 M NaOH. To the solubilized protein a volume of 30 μL sample buffer (100 mM Tris/HCl at pH 6.7; 20% w/v glycerol; 2.0% w/v SDS; 6 M urea; 100 mM dithiothreitol; and 0.1% w/v bromophenol blue) was added, and the mixture was heated to 80 °C prior to resolution by electrophoresis on an SDS/polyacrylamide gel (10% w/v) [28]. Following electrophoresis, the resolved proteins were transferred to a nitrocellulose filter according to a standard protocol. Detection of the immobilized proteins was performed by adding a primary antibody against Mls1p (diluted 1:2000) or peroxisomal catalase A (Cta1p, diluted 1:1000) [27], followed by application of the enhanced chemiluminescence (ECL) system from Pierce (Super Signal West Pico Chemiluminescent Substrate; no. 34083). Determination of protein concentration was performed as described [29].

**Purification of tagged Mls1p and generation of anti-Mls1p Ig**

To obtain pure protein for generating an antibody against Mls1p, the pQE-32 expression system (Qiagen Inc., Valencia, CA, USA) was used. A DNA fragment encoding the
C-terminal 308 amino acids (out of a total of 554) was used to express a soluble His-tagged protein (His$_6$-Mls1p) in bacterial cells. Cell lysates were subjected to affinity chromatography using a Ni$^{2+}$-containing Sepharose 6B column (Pharmacia), and protein was purified to near homogeneity using a Ni-nitrilotriacetic acid Spin Kit (Qiagen). SDS/PAGE revealed a protein band with an apparent molecular mass of 38 000, which corresponded to the deduced size of the His$_6$-Mls1p truncation (not shown).

A fraction of a purified His$_6$-Mls1p was immobilized on a membrane and subjected to tryptic digestion, and HPLC-purified peptide fragments were microsequenced. The sequences obtained, GVHAMGGMAAQIPIK and ATPTDLSK, corresponded to the respective deduced residues 334–348 and 546–553 of Mls1p, confirming the identity of the purified recombinant protein. The same purified protein (100 µg) in combination with complete Freund’s adjuvant (3 mL total volume) was used to immunize rabbits (approved by the Ethics Committee of the University of Vienna). This was followed by three additional booster injections. After ammonium sulfate precipitation and DEAE-ion exchange of the antiserum, antibody was used for immunoblotting. For immunoelectron microscopy, the antibody preparation was subjected to affinity purification using membrane-immobilized soluble protein extracts obtained from yeast cells over-expressing native Mls1p.

RESULTS

The subcellular location of Mls1p

Malate synthase 1 terminates with an SKL tripeptide representing a peroxisomal targeting signal PTS1 [5,15]. To determine whether Mls1p is indeed a peroxisomal protein, electron microscopy was performed using an anti-Mls1p antibody that was generated against a recombinant protein comprising the C-terminal 308 amino acids of Mls1p. Although it cannot be entirely ruled out that the antibody used additionally cross-reacts with Dal7p, which is 81% identical to Mls1p and also ends with SKL, expression of Dal7p in cells grown in the presence of ample nitrogen was considered to be unlikely as transcription of the corresponding $DAL7$ gene is tightly repressed under these medium conditions [5].

Purified antibody was applied to a filter containing soluble protein extracts obtained from wild-type and mls1D cells that were propagated in rich medium supplemented with ethanol. This resulted in a protein band with a molecular mass of 62 000 in the lane with the wild-type extract that was absent from the lane corresponding to the mls1D mutant (arrow; Fig. 1A), thereby confirming the specificity of the antibody. Application of the antibody to thin sections of wild-type cells grown on oleic acid medium

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Fig. 1. SKL is required to direct Mls1p to the peroxisomes under oleic acid-medium conditions. (A) Specificity of the anti-Mls1p antibody. Extracts from homogenized wild-type (GA1-8C) and mls1D yeast (JR85) strains were immobilized on a membrane to which anti-Mls1p Ig was applied. A single protein band with a molecular mass of 62 000 is seen only in the lane representing the wild-type extract (arrow). (B) Immunoelectron micrograph of a wild-type yeast cell expressing native Mls1p from the chromosomal locus (GA1-8C). Gold particles representing Mls1p in the matrix of peroxisomes are indicated (arrows). l, lipidic inclusion; m, mitochondrion; n, nucleus; and p, peroxisome. The bar is 1 µm. (C) Micrograph of an mls1D mutant over-expressing an SKL-less Mls1p (KM15). Gold particles (marked with arrows) are seen in the nucleus, cytoplasm, and in some case also in mitochondria, peroxisomes, and lipidic inclusions. The bar and letters are equivalent to those in (B).
resulted in the decoration of peroxisomes (Fig. 1B). This result lent credence to the suggested peroxisomal location of Mls1p based on a GFP-Mls1p green fluorescent protein reporter expressed in cells grown on oleic acid [30]. Use of this antibody with thin sections of an otherwise isogenic mls1Δdal7Δ strain over-expressing an SKL-less Mls1p variant (Mls1pΔSKL; strain KM15) on oleic acid revealed gold particles decorating both the nucleus and cytosol (Fig. 1C), which was consistent with a noncompartmentalized antigen. The results indicated that the SKL tripeptide was important for peroxisomal targeting.

Peroxisomal import of Mls1p depends on oleic acid

The glyoxylate cycle is essential for cell growth on media supplemented with nonfermentable carbon sources not requiring peroxisomes for their metabolism, e.g. ethanol or acetate, and is physiologically functional in mutant pex cells lacking a normal peroxisomal compartment [19]. This raised the issue of whether Mls1p is compartmentalized during growth of cells under such medium conditions. To examine the subcellular location of malate synthase 1 in cells grown on ethanol, a GFP reporter was constructed that was extended with the C-terminal 274 amino acids of Mls1p (out of a total of 554), including the terminal SKL. Expression of this GFP-Mls1p was compared to that of a control GFP extended solely by SKL (GFP-SKL). GFP-SKL has been amply shown before to be imported into the peroxisomes of wild-type cells, but to remain cytosolic in pex mutant cells devoid of functional peroxisomes [22,31]. The results demonstrated that living yeast cells expressing either GFP-Mls1p or GFP-SKL on oleic acid exhibited bright, closely bunched fluorescent points (Fig. 2, upper panels). On the other hand, in cells grown on ethanol, the punctate pattern of fluorescence due to GFP-SKL was less diffuse, whereas fluorescence due to GFP-Mls1p was altogether diffuse (Fig. 2, lower panels). This indicated that unlike the situation with GFP-SKL, which was targeted to peroxisomes in cells grown under both medium conditions, compartmentalization of GFP-Mls1p into peroxisomes depended on cell growth on oleic acid medium.

To reinforce the evidence for the differential subcellular location of Mls1p, cellular fractionation was used. Fractions were prepared from ethanol-grown cells that contained import-competent peroxisomes as they could compartmentalize GFP-SKL efficiently (Fig. 2). Lysates of homogenized wild-type cells were spun to yield an organellar pellet consisting of mitochondria and peroxisomes, and a cytosolic supernatant. Equal fractions of each of the protein preparations (10% of total vol) were immobilized on replicate membranes which were probed with anti-malate synthase (α-Mls1p) or anti-catalase A (α-Cta1p) Ig. Molecular mass markers (kDa) are indicated to the left.

Fig. 2. Subcellular localization of GFP-Mls1p. Oleic acid-grown BJ1991 cells transformed with GFP-Mls1p or GFP-SKL were monitored by direct fluorescence microscopy. Punctate fluorescence indicated presence of GFP in peroxisomes. The diffuse fluorescence seen in ethanol-grown cells expressing GFP-Mls1p was commensurate with a cytosolic localization of the reporter protein. Nomarski images corroborated the integrity of the cells examined.

Targeting of Mls1p to peroxisomes is advantageous for growth on oleic acid

Two steps of the glyoxylate cycle take place in the cytosol: the splitting of isocitrate into succinate and glyoxylate, and the dehydrogenation of malate to oxaloacetate (Scheme 1).
However, the intervening activity undertaken by Mls1p, i.e. formation of malate from glyoxylate and acetyl-CoA, occurs in the peroxisomes when cells are grown on oleic acid. This prompted the question of whether there is any advantage to cells targeting Mls1p to peroxisomes, as by doing so cells partition the enzyme reactions to either side of the organellar membrane. To examine the requirement for compartmentalizing Mls1p, yeast mls1Δ cells (KM12) and strains expressing native Mls1p or Mls1pΔSKL from the chromosomal locus (strains KM13 and KM15) were grown on solid fatty acid medium. The medium used also contained Tween 80, which acted to disperse the fatty acids but was also a poor carbon source. Hence, mutant cells often grow to some extent on these plates but transparent zones in the opaque medium around regions of cell growth indicate utilization of the fatty acid substrate [25]. Application of serial dilutions of cell cultures (BJ1991, KM12, KM13) to this medium showed that the mls1Δ mutant was unable to form a clear zone (Fig. 4A). On the other hand, despite representing a strictly cytosolic protein, Mls1pΔSKL appeared to overcome the mutant phenotype (Fig. 4A).

To examine whether a cytosolic malate synthase was as efficient as a peroxisomal one for maintaining a functional glyoxylate cycle on oleic acid, liquid growth assays were conducted. The results showed that the growth rate of cells expressing wild-type Mls1p was higher compared with those producing Mls1pΔSKL (Fig. 4B). Vital counts based on this assay served to confirm that although the compartmentalization of malate synthase was not strictly essential, it was advantageous for cells to grow on oleic acid (Fig. 4C). The greater sensitivity of liquid growth assays on oleic acid compared with solid medium has been previously reported [32].

As a control, cells were streaked on ethanol, acetate, or glucose media (Fig. 5A). The results demonstrated that the mls1Δ mutant failed to grow on ethanol or acetate. However, expression of either of the two Mls1p constructs complemented the mls1Δ mutant phenotype on these media. Growth assays in liquid medium supplemented with ethanol similarly showed that although mls1Δ cells were unable to multiply, those cells expressing malate synthase in any form, i.e. Mls1p or Mls1pΔSKL, grew abundantly (Fig. 5B). This indicated that a constitutively cytosolic Mls1p was sufficient for cells to maintain the metabolite flux through the glyoxylate cycle during growth on nonfermentable carbon sources other than fatty acids.

**DISCUSSION**

The requirement for the compartmentalization of the yeast glyoxylate cycle into peroxisomes has been put into question in light of chronicled observations of growth of *S. cerevisiae* pex mutants devoid of functional peroxisomes on ethanol [19]. In addition, pex mutants have also been demonstrated to undergo normal meiosis and sporulation in liquid acetate medium [33], processes which similarly require a functional glyoxylate cycle [34]. However, as pex mutants fail to grow or sporulate in liquid oleic acid medium [33], the issue of the partitioning of the glyoxylate cycle in cells grown under fatty acid-medium conditions has hitherto remained open.

We showed here that one of the key glyoxylate-cycle enzymes, Mls1p, was cytosolic in cells grown on ethanol, whereas in cells grown on oleic acid Mls1p was peroxisomal. This is the first time that the targeting of an SKL-terminating protein into peroxisomes is shown to be different depending on the growth conditions. A previous study on the subcellular distribution of AKL-terminated...
aspartate aminotransferase Aat2p demonstrated that this protein was compartmentalized in cells grown on oleic acid, but remained in the cytosol of glucose-grown cells [35]. However, under these latter conditions peroxisomes are very few due to catabolite repression [36,37], whereas on ethanol peroxisomes are not only more readily detectable, but are additionally import competent (Fig. 2). This means that unlike the situation with Aat2p which essentially has no target compartment in cells grown on glucose, Mls1p was selectively retained in the cytosol of cells propagated on ethanol. Interestingly, the C-termini of both Mls1p and Aat2p contain acidic amino-acid residues at the 5th-last position with respect to the terminal residue (DLSKL in Mls1p and EISKL in Aat2p), which is unusual at this position [21]. The significance of this similarity is currently being addressed.

Demonstration of the cytosolic location of Mls1p in wild-type cells grown on ethanol completes the picture of the extra-peroxisomal location of the glyoxylate cycle in yeast grown on carbon sources other than fatty acids. The only other key enzyme unique to the glyoxylate cycle, Icl1p, is also extra-peroxisomal [4], as are the other enzymes essential for the glyoxylate cycle (Scheme 1) including mitochondrial citrate synthase encoded by CIT1 (and possibly also by CIT3), cytosolic Mdh2p, and extra-peroxisomal Aco1p.

As mentioned previously, malate synthase catalyses the formation of malate from glyoxylate and acetyl-CoA, the source of the latter being either peroxisomal when breaking down fatty acids, or cytosolic when extra-cellular two-carbon substrates are used. Although not strictly essential, the peroxisomal localization of malate synthase 1 appears to be advantageous for cells growing on oleic acid, in that acetyl-CoA production and utilization are thereby intimately compartmentalized together to increase efficiency. Future work on the entry of glyoxylate into peroxisomes will help elucidate how the glyoxylate cycle proceeds across an organellar membrane in cells grown on oleic acid. In addition, solution of the crystal structure of Mls1p could also turn out to be helpful in elucidating whether the protein’s selective import into peroxisomes might have something to do with the exposure of the C-terminal SKL tripeptide for making contact with the cognate receptor Pex5p.

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