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Mitochondrial transporters involved in oleic acid utilization and glutamate metabolism in yeast

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Abstract

Utilization of fatty acids such as oleic acid as sole carbon source by the yeast *Saccharomyces cerevisiae* requires coordinated function of peroxisomes, where the fatty acids are degraded, and the mitochondria, where oxidation is completed. We identified two mitochondrial oxodicarboxylate transporters, Odc1p and Odc2p, as important in efficient utilization of oleic acid in yeast [Tibbetts et al., Arch. Biochem. Biophys. 406 (2002) 96–104]. Yet, the growth phenotype of *odc1Δodc2Δ* strains indicated that additional transporter(s) were also involved. Here, we identify two putative transporter genes, *YMC1* and *YMC2*, as able to suppress the *odc1Δodc2Δ* growth phenotype. The mRNA levels for both are elevated in the presence of glycerol or oleic acid, as compared to glucose. Ymc1p and Ymc2p are localized to the mitochondria in oleic acid-grown cells. Deletion of all four transporters (quad mutant) prevents growth on oleic acid as sole carbon source, while growth on acetate is retained. It is known that the glutamate-sensitive retrograde signaling pathway is important for upregulation of peroxisomal function in response to oleic acid and the oxodicarboxylate α-ketoglutarate is transported out of the mitochondria for synthesis of glutamate. So, citric acid cycle function and glutamate synthesis were examined in transporter mutants. The quad mutant has significantly decreased citrate synthase activity and whole cell α-ketoglutarate levels, while isocitrate dehydrogenase activity is unaffected and glutamate dehydrogenase activity is increased 10-fold. Strains carrying only two or three transporter deletions exhibit intermediate affects. ^{13}C NMR metabolic enrichment experiments confirm a defect in glutamate biosynthesis in the quad mutant and, in double and triple mutants, suggest increased cycling of the glutamate backbone in the mitochondria before export. Taken together these studies indicate that these four transporters have overlapping activity, and are important not only for utilization of oleic acid, but also for glutamate biosynthesis.

Keywords: Mitochondria; Transport; Fatty acid; Glutamate; Citric acid cycle; Peroxisome; Yeast

Efficient coordination of functions in the various organelles of eukaryotes is important for a myriad of processes from metabolism to gene expression. In the yeast *Saccharomyces cerevisiae*, utilization of long chain fatty acids such as oleic acid requires the collective functions of peroxisomes and mitochondria. Fatty acids are first shortened via β-oxidation in the peroxisome. These metabolites then enter the mitochondria for further oxidation through the citric acid cycle and energy production via oxidative phosphorylation [1–3]. Adaptation of *S. cerevisiae* to oleic

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acid as carbon source, therefore, involves effective upregulation of both mitochondrial and peroxisomal function [4]. As with other non-fermentable carbon sources, glucose repression of genes encoding proteins involved in the citric acid cycle and respiration must be released, which requires a number of genes including *SNFs* and the *HAP* complex [5,6]. Additionally, in the case of fatty acid carbon sources like oleic acid, peroxisomal biogenesis, and expression of β-oxidation enzymes are induced. The promoters of many oleic acid-sensitive genes contain oleate response elements (OREs)[2] which bind and are activated by transcription factors Oaf1p and Pip2p [4,7].

Over and above its bioenergetic function, the mitochondrial citric acid cycle also provides biosynthetic precursors such as α-ketoglutarate, which is used to synthesize glutamate. Among the pathways of mitochondria-nucleus communication, which are vital to synchronize gene expression in these two organelles [8] is the retrograde or RTG pathway; this signals the nucleus in the event of respiratory dysfunction to maintain the cellular supply of glutamate [9]. RTG signaling involves transcription factors which positively regulate gene expression in alternative pathways to provide substrates, including citrate, acetyl-CoA and oxaloacetate, and restore α-ketoglutarate and glutamate synthesis. Activity of the glyoxylate cycle in peroxisomes is induced, characterized by elevated citrate synthase 2 (*CIT2*) transcription, as is peroxisome proliferation [9,10]. Interestingly, oleic acid induction of *POX1* (encoding peroxisomal acyl-CoA oxidase) and *CTA1* (encoding peroxisomal catalase), which are required for yeast to utilize fatty acids as sole carbon source, is also dependent upon RTG signaling [9,10], demonstrating an indirect connection between fatty acid β-oxidation and glutamate biosynthesis.

Our studies have been aimed at the identification of genes required for assimilation of long chain oleic acid as a carbon source and/or the coordination of peroxisome and mitochondrial function by examining yeast mutants that retain the ability to utilize acetate, which requires mitochondrial respiration, while losing the ability to utilize oleic acid, which requires peroxisomal β-oxidation and mitochondrial respiration [11–13]. Among the genes, we identified as necessary for efficient growth on oleic acid were *ODC1* and *ODC2*, which encode for homologous mitochondrial transporters reported to catalyze the counter-exchange of 2-oxoglutarate (α-ketoglutarate), 2-oxoadipate (α-ketoadipate) or malate across reconstituted membranes [13,14]. Expression of *ODC1* mRNA and Odc1p is significantly elevated in response to oleic acid in comparison to glucose or glycerol via a mechanism that does not require the oleic acid-responsive transcription factors Oaf1p and Pip2p. Further, yeast strains carrying *odc1Δ* and *odc2Δ* alleles exhibit a temperature-sensitive inability to grow on oleic acid at 36 °C. Growth of *odc1Δodc2Δ* strains at 30 °C, however, is normal. The temperature-sensitive phenotype of *odc1Δodc2Δ* strains led us to propose that a third transporter with similar activity is present in yeast, but is unable to compensate for the loss of *ODC1* and *ODC2* at elevated temperature [13].

The present studies were conducted in an effort to identify this putative transporter. A multicopy suppressor screen resulted in the identification of two homologous genes predicted to encode mitochondrial transporters, *YMC1* [15] and *YMC2* [16], as able to suppress the oleic acid growth defect of strains lacking *ODC1* and *ODC2* [17]. Examination of growth characteristics of mutants, along with expression studies of *YMC1* and *YMC2* and localization of the YMC-encoded proteins, indicates that these genes encode for mitochondrial transporters with function(s) overlapping *ODC1* and *ODC2*. Furthermore, since α-ketoglutarate is a preferred substrate reported for Odc1p and Odc2p [14], glutamate metabolism was examined in mutants carrying transporter mutations. The results reveal that, in addition to their observed necessity for utilization of oleic acid, these transporters are important for glutamate metabolism in yeast.

Materials and methods

Yeast strains and media

Genotypes of yeast strains used in these studies are listed in Table 1. The fat39 and *odc1Δodc2Δ* (ATY 6) mutant strains were used in our previous studies [11]. The *ymc1Δ ymc2Δ* (CSY1) strain was obtained by crossing Research Genetics (Huntsville, AL) strains 5477 (MATa *his3 leu2 met15 ura3 ymc1Δ*::*G418*) and 13243 (*ymc2Δ*, see Table 1). The triple and quad mutants were from a cross of CSY4 (MATα *his3 leu2 met15 ura3 ymc1Δ*::*G418 ymc2Δ*::*G418*), a sibling spore to CSY1, and CSY6 (MATα *his3 leu2 lys2 ura3 odc1Δ*::*G418 odc2Δ*::*G418*). CSY6 was obtained by mating-type switch of ATY6 [18] using plasmid pAAB250-*HO-URA3*, a kind gift of Dr. Arlen Johnson (University of Texas-Austin). Eviction of plasmid and *URA3* was induced by growth on 5-fluoroorotic acid (5-FOA) medium containing 0.7% yeast nitrogen base, 0.1% 5-FOA (Diagnostic Chemicals Limited), 2% glucose, 50 mg/L uracil and amino acids. The presence of disrupted alleles was confirmed by polymerase chain reaction (PCR) amplification using primers flanking each locus.

[2] *Abbreviations used:* OREs, oleate response elements; *CIT2*, citrate synthase 2; PCR, polymerase chain reaction; ATCC, American Type Culture Collection; RT-PCR, reverse transcription polymerase chain reaction; EDTA, ethylene diamine tetraacetic acid; GlDH, Glutamate dehydrogenase; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); GlDH, glutamate dehydrogenase; CAC, citric acid cycle; CS, citrate synthase; IDH, isocitrate dehydrogenase.