A Mitochondrial Pyruvate Dehydrogenase Bypass in the Yeast Saccharomyces cerevisiae*

(Received for publication, December 14, 1998, and in revised form, May 10, 1999)

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Spheroplasts of the yeast Saccharomyces cerevisiae oxidize pyruvate at a high respiratory rate, whereas isolated mitochondria do not unless malate is added. We show that a cytosolic factor, pyruvate decarboxylase, is required for the non-malate-dependent oxidation of pyruvate by mitochondria. In pyruvate decarboxylase-negative mutants, the oxidation of pyruvate by permeabilized spheroplasts was abolished. In contrast, deletion of the gene (PDA1) encoding the E1α subunit of the pyruvate dehydrogenase did not affect the spheroplast respiratory rate on pyruvate but abolished the malate-dependent respiration of isolated mitochondria. Mutants disrupted for the mitochondrial acetaldehyde dehydrogenase gene (ALD7) did not oxidize pyruvate unless malate was added. We therefore propose the existence of a mitochondrial pyruvate dehydrogenase bypass different from the cytosolic one, where pyruvate is decarboxylated to acetaldehyde in the cytosol by pyruvate decarboxylase and then oxidized by mitochondrial acetaldehyde dehydrogenase. This pathway can compensate PDA1 gene deletion for lactate or respiratory glucose growth. However, the codisruption of PDA1 and ALD7 genes prevented the growth on lactate, indicating that each of these pathways contributes to the oxidative metabolism of pyruvate.

Pyruvate is a key intermediate in sugar metabolism. Three major pathways of pyruvate metabolism in the yeast Saccharomyces cerevisiae have been described (for a review, see Ref. 1) (Fig. 1). During fermentative growth, pyruvate is decarboxylated into acetaldehyde by pyruvate decarboxylase, which is, in its turn, reduced into ethanol in the cytosol by ADH1, one of the four known alcohol dehydrogenase isoenzymes (2, 3). This sequence of reactions allows the reoxidation of NADH, which is produced at the level of the glyceraldehyde-3-phosphate dehydrogenase. During respiratory metabolism, pyruvate can enter the mitochondria by a specific carrier (4, 5) and is decarboxylated and oxidized into acetyl-CoA by pyruvate dehydrogenase, a multienzyme complex located in the matrix (6). In addition, a pyruvate dehydrogenase bypass located in the cytosol converts pyruvate into acetyl-CoA by the action of the following enzymes: pyruvate decarboxylase (7), cytosolic acetaldehyde dehydrogenase (8, 9), and acetyl-CoA synthetases (10, 11). Acetyl-CoA synthesized in the cytosol is either directly used for the biosynthetic pathways or enters the mitochondria via the carnitine acyltransferase system (12, 13). It has been proposed that this system works unidirectionally; i.e. acetyl-CoA can only move from the outside into inside (13). In contrast, direct oxidative decarboxylation of pyruvate into acetyl-CoA by the pyruvate dehydrogenase complex does not require ATP hydrolysis, since the energy required for the thioester formation is provided by oxidation of the carbonyl into carboxyl groups (Fig. 1). It is generally assumed that in wild-type S. cerevisiae grown under glucose limitation, the pyruvate dehydrogenase complex is primarily responsible for pyruvate catabolism (14). The pyruvate dehydrogenase bypass by cells reduces the net ATP yield. A deletion of the gene encoding the enzyme E1α of the pyruvate dehydrogenase complex leads to complete loss of pyruvate dehydrogenase activity. However, in aerobic glucose-limited chemostat cultures of this strain, pyruvate metabolism has been shown to be fully respiratory (15–17). In S. cerevisiae, the anaerobic synthesis of oxaloacetate from pyruvate is catalyzed by pyruvate carboxylase, an exclusively cytosolic enzyme, in contrast to many higher organisms in which this enzyme is mitochondrial (14, 18).

We recently observed that permeabilized yeast spheroplasts oxidized pyruvate at a high rate, while isolated mitochondria did not (19). This respiration is now shown to be due to the oxidation by mitochondria of acetaldehyde produced in the cytosol; moreover, the pathway can compensate for the absence of functional mitochondrial pyruvate dehydrogenase complex. Pyruvate decarboxylase, the key enzyme in alcoholic fermentation is operative under conditions in which alcoholic fermentation is absent. We propose that the acetaldehyde produced by this enzyme is oxidized by the mitochondrial acetaldehyde dehydrogenase, allowing the reduction of NAD⁺ inside the matrix. The acetate produced can be converted into acetyl-CoA in the cytosol or excreted in the culture medium. The role of this pathway in ethanol metabolism is discussed.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions—The S. cerevisiae strains used in this study were either the diploid PS194 (Yeast Foam, from the Slonimski laboratory) or the haploids listed in Table I. They were cultivated in rich yeast peptone medium (1% yeast extract, 2% bactopeptone, 0.1% potassium phosphate, 0.12% ammonium sulfate, pH 5) or yeast nitrogen base medium (0.17% yeast nitrogen base, 0.1% potassium phosphate, 0.5% ammonium sulfate, pH 5) containing auxotrophic supplements for growth. Carbon sources used at a concentration of 2% were lactate, ethanol, glycerol, and glucose. Cells were grown in a New Brunswick incubator at 28 °C, and they were harvested in the logarithmic growth phase (20). Growth rates were monitored by turbidity measurements.

Disruption of PDA1 Gene from RY271 Strain—PDA1 was amplified by polymerase chain reaction using the primers 5'-GGTGCCAAAT-AGCTTCTCT-3' and 5'-TTGCCAAAATGACTCGGGA-3' and se-
enzymes: glucose transporter (reaction 1), pyruvate dehydrogenase (reaction 2), cytosolic acetaldehyde dehydrogenase (reaction 3), acetyl-CoA synthetase (reaction 4), cytosolic alcohol dehydrogenases (reaction 5), mitochondrial pyruvate carrier (reaction 6), pyruvate dehydrogenase complex (reaction 7), carnitine acetyltransferase (reaction 8), and pyruvate carboxylase (reaction 9). TCA cycle, tricarboxylic cycle.

**Preparation of Spheroplasts and Mitochondria**—Spheroplasts were prepared according to the enzymatic procedure described previously (20, 22). Spheroplasts were suspended at 1 mg/ml in buffer containing 1 m sorbitol, 0.5 mM EGTA, 2 mM MgSO₄, 1.7 mM NaCl, 10 mM KHzPO₄, and 1% bovine serum albumin, pH 6.8, and permeabilized by nystatin according to Ref. 19. Yeast mitochondria (S. cerevisiae) were prepared as described in Ref. 20 and suspended in a medium containing 0.65 m mannitol, 0.36 mM EGTA, 10 mM Tris-maleate, 5 mM Tris-phosphate, pH 6.8 (23).

**Protein Fractionation**—The postmitochondrial supernatant was centrifuged for 60 min at 100,000 × g, and the new one was submitted to the following steps of protein fractionation. Proteins were precipitated at 65% ammonium sulfate saturation. The pellet was suspended in the buffer containing 10 mM potassium phosphate, 1 mM EDTA, 1 mM β-mercaptoethanol, pH 6.8, and dialyzed overnight against this buffer. The dialyzed fraction was loaded on a Mono Q column fast protein liquid chromatography system (MonQ-HR 5/5, Amersham Pharmacia Biotech) that had been equilibrated with this buffer. The protein was eluted with a linear gradient of 0–1 m KCl. The concentrated active fraction (defined under “Results”) was loaded on a gel filtration Superose-12 HR 10/30 column. Elution was carried out with the buffer without added KCl. Fractions containing proteins were stored at −20 °C. All steps were performed at 4 °C and in the presence of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM aminocaproic acid, and 10 mM para-aminobenzamidine).

**Other Methods**—Oxygen consumption by isolated mitochondria and spheroplasts suspended in their isolation medium was measured at 28 °C in a 1.5-mL thermostatically controlled chamber equipped with a Clark oxygen electrode (Gibson) connected to a microcomputer giving an on-line display of rate values.

Enzyme assays were carried out at 30 °C with an Eppendorf spectrophotometer at 334 nm. Pyruvate decarboxylase (EC 4.1.1.1) activity was assayed according to Ref. 14, and pyruvate dehydrogenase activity (EC 1.2.4.1) was assayed according to Ref. 24. Mitochondrial acetaldehyde dehydrogenase (EC 1.2.1.5) was assayed according to Jacobson et al. (25, 26). Malate in spheroplast extract was determined enzymatically with a malic enzyme (EC 1.1.1.83) according to Bergmeyer (27). Proteins were estimated by the biuret method.

**Chemicals**—Pyruvate decarboxylase and alcohol dehydrogenase from brewers yeast were purchased from Sigma. Malic enzyme and all other substrates and auxotrophic supplements were obtained from Sigma.

**TABLE I**

| Genotypes and sources of S. cerevisiae strains |
|------------------------------------------------|
| Strain | Genotype | Activity lacking | Sources |
| YSH6.36-3D | MATa leu2–3/112ura3–52trp1–92 | None (parental strain) | Ref. 28 |
| YSH4.136–2D | MATa trp192leu23/112ura352 pdc1::LEU2 pdc5::URA3 | Pyruvate dehydrogenase | Ref. 28 |
| T2–3D | HO/HO PDA1/PDA1 | None (parental strain) | Ref. 17 |
| T2–3C | HO/HO pda1::Tn5ble/pda1::Tn5ble | Pyruvate dehydrogenase | Ref. 17 |
| RY601 | MATa ura3–52lys2–801 ade2–101 trp1–Δ63 his3–Δ200 leu2–Δ1 | None (parental strain) | Ref. 31 |
| RY271 | MATa ura3–52lys2–801 ade2–101 trp1–Δ63 his3–Δ200 leu2–Δ1 aad7::HIS3 | Mitochondrial acetaldehyde dehydrogenase | Ref. 31 |
| RY271-BS1 | MATa ura3–52lys2–801 ade2–101 trp1–Δ63 his3–Δ200 leu2–Δ1 aad7::HIS3 pda1::URA3 | Mitochondrial acetaldehyde dehydrogenase and pyruvate dehydrogenase | This work |

**TABLE II**

Comparison of respiratory rates between isolated mitochondria and permeabilized spheroplasts from PS194 strain incubated with different substrates.

Mitochondria (0.5 mg/ml) and spheroplasts (1 mg/ml) were incubated in the medium as described under “Experimental Procedures.” Permeabilization of spheroplasts was performed by incubation with 20 μg/ml nystatin for 10 min. Respiratory rates were measured after the addition of various substrates. All substrates were used at 10 mM except for 10 mM ethanol. In some samples, 2 mM ADP was added. To compare the relative activities of spheroplasts and isolated mitochondria, the respiratory values (+ ADP) were expressed as a percentage of ethanol respiratory rate. Experimental results are the average ± S.D. of measurements with at least four independent preparations.

| Substrates | Permeabilized spheroplasts | Isolated mitochondria | Respiratory rate |
|------------|---------------------------|-----------------------|----------------|
|            | −ADP | −ADP | ADP | ADP |                  |                  |                |
| Ethanol    | 78 ± 5 | 144 ± 6 | 100 | 227 ± 20 | 496 ± 45 | 100 |
| Pyruvate   | 65 ± 4 | 110 ± 9 | 76 | 14 ± 4 | 15 ± 4 | 4 |
| Malate     | 6 ± 1 | 6 ± 1 | 4 | 14 ± 3 | 12 ± 3 | 2 |
| Pyruvate + malate | 87 ± 4 | 148 ± 8 | 102 | 66 ± 8 | 83 ± 15 | 20 |
RESULTS

Comparison of Respiratory Metabolism between Mitochondria and Permeabilized Spheroplasts—Table II compares the respiration rates on different substrates between isolated mitochondria and permeabilized spheroplasts. ADP stimulated the respiration rate on spheroplasts as it did with isolated mitochondria, indicating that nystatin did not uncouple oxidative phosphorylation. The respiratory values in the presence of ADP were also expressed as a percentage of ethanol respiratory rate. Indeed, the respiration of permeabilized spheroplasts on ethanol was due to oxidation inside the mitochondrial matrix, as for isolated mitochondria, since cytosolic NADH was diluted into the medium. The main result was the high respiration rate on pyruvate observed with permeabilized spheroplasts as compared with that observed with isolated mitochondria. It is known that mitochondria do not oxidize pyruvate unless malate is added, since the oxaloacetate produced by malate dehydrogenase reacts with acetyl-CoA, a potent inhibitor of pyruvate dehydrogenase (6). The spheroplast extract contained 30 ± 5 μM malate (determination on four different preparations), and the apparent affinity constant of the stimulation by malate of pyruvate respiration on isolated mitochondria was 125 ± 20 μM of malate. Therefore, it appears that the medium did not contain enough malate to explain the respiration on pyruvate. Moreover, Table II shows that malate addition stimulated the respiration on pyruvate, suggesting two kinds of spheroplast respiration on pyruvate: one dependent on malate and another not.

Characterization of the Molecule Involved in the Oxidation of the Pyruvate in Permeabilized Spheroplasts—First, we tried to determine the step of mitochondrial preparation corresponding to the loss of the factor involved in respiration on pyruvate. This step corresponded to the first centrifugation (12,000 × g, 10 min), which led to the separation of the mitochondrial fraction from the cytosolic one. The cytosolic factor was purified as described under “Experimental Procedures,” and the activity was measured as its capacity to induce the respiration of isolated mitochondria on pyruvate. As shown in Fig. 2, the active fraction copurified with the pyruvate dehydrogenase activity. The electrophoretic gel pattern of the partially purified active fraction showed a major band with an apparent molecular mass of 65 kDa, corresponding to the monomer of pyruvate dehydrogenase (data not shown). Moreover, respiration of isolated mitochondria with pyruvate as the sole substrate was induced by addition of commercial pyruvate dehydrogenase: a respiration of 367 ± 35 nanoatoms of oxygen/min/mg of protein was determined in the presence of 2 mM ADP and 1 unit/ml enzyme, a value identical to the one obtained with a nonlimiting amount of ethanol (four independent determinations). These results could suggest that the pyruvate was first decarboxylated in the cytosol and that the acetaldehyde oxidized to acetate in the mitochondria. This interpretation was confirmed by the use of different mutants.

Growth Characteristics of Wild-type and Mutant Strains—Two structural genes, PDC1 and PDC5, encode pyruvate decarboxylase in yeast (28). The enzyme activity is poorly affected or not affected by deletion of either of these genes but is undetectable in mutants lacking both PDC1 and PDC5 (28, 29). It is known that the double mutant cannot grow in rich medium with glucose, even in glucose-limited chemostat culture at a low dilution rate corresponding to a respiratory metabolism (30). We also observed that the double mutant was unable to grow on lactate as a carbon source unless acetate or ethanol were added (Table III) as proposed previously for culture on glucose by Flikweert et al. (30), who proposed that this inability of the pdc1Δpdc5Δ mutant to grow on glucose is due to the need for C-2 compounds in lipid biosynthesis (30).

In the mutant T2–3C, PDA1 encoding the E1α subunit of the pyruvate dehydrogenase is disrupted (pda1Δ). The mutation leads to a complete loss of pyruvate dehydrogenase activity (17). In agreement with a previous report on glucose-limited chemostat cultures (15), this mutation had no significant effect on the specific growth rate on the different nonfermentable carbon sources tested (Table III).

![FIG. 2. Characterization of the cytosolic active fraction from S. cerevisiae. Broken spheroplasts were centrifuged at 12,000 × g, and proteins of the supernatant were fractionated as described under “Experimental Procedures.” Solid line, absorbance at 280 nm; ○, pyruvate dehydrogenase activity; ■, respiratory rate of isolated mitochondria (0.5 mg/ml protein) on pyruvate (10 mM) in the presence of 100 μl of the different fractions for a total volume of 3 ml.](image-url)

### TABLE III

Growth rates of yeast strains with various carbon sources

| Strains and relevant genotype | Ethanol (YP<sup>a</sup>, YNB<sup>a</sup>) | Lactate (YP<sup>a</sup>, YNB<sup>a</sup>) | Glucose (YP<sup>a</sup>, YNB<sup>a</sup>) |
|-----------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| YSH6.36–3D (PDC1 PDC5)      | 3.15 ND<sup>a</sup>                  | 4                                    | 5                                    |
| YSH4.136.2D (pdc1::LEU2 pdc5::URA3) | 3.15 ND<sup>a</sup>                  | NG<sup>d</sup>                       | NG<sup>d</sup>                       |
| T2–3D (pda1::Tn5::ble/pda1::Tn54::ble) | 3                                  | 4                                    | 2                                    |
| T2–3C (pda1::Tn5::ble/pda1::Tn54::ble) | 3                                  | 4                                    | 2                                    |
| RY601 (ALD7)                 | 5                                   | 4                                    | 3.10                                 |
| RY271 (ald7::HIS3)           | NG                                   | NG                                   | 3.10                                 |
| RY271-BS1 (ald7::HIS3 pda1::URA3) | NG                                   | 5                                    | 3.30                                 |

<sup>a</sup> Culture growth rates were measured during logarithmic growth.

<sup>b</sup> Rich yeast peptone (YP) medium and minimal yeast nitrogen base (YNB) were used for cultivation with various carbon sources.

<sup>c</sup> ND, not determined.

<sup>d</sup> NG, no growth after 35 h of incubation.
Comparison of respiratory rates of the permeabilized spheroplasts with various substrates between mutants and their parental strains

Respiratory activities were measured in the presence of 2 mM ADP; the other conditions are described in legend of Table II. Experimental results are the average ± S.D. of measurements with at least three independent spheroplast preparations.

| Strains                  | Ethanol  | Succinate | Pyruvate | Malate | Pyruvate + malate |
|-------------------------|----------|-----------|----------|--------|------------------|
| YSH6.36–3 (PDC1 PDC5)   | 125 ± 11 | 130 ± 9   | 54 ± 6   | 7 ± 2  | 64 ± 10          |
| YSH4.136–2D (pdc1::LEU2 pdc5::URA3) | 112 ± 9  | 91 ± 11   | 7 ± 1    | 8 ± 3  | 40 ± 6           |
| T2–3D (PDA1 /PDA1)      | 150 ± 10 | 104 ± 7   | 138 ± 10 | 5 ± 2  | 162 ± 10         |
| T2–3C (pda1::Tn5ble/pda1::Tn5ble) | 120 ± 8  | 100 ± 4   | 124 ± 9  | 3 ± 1  | 120 ± 8          |
| RY601 (ALD7)            | 104 ± 4  | 72 ± 6    | 52 ± 7   | 4 ± 1  | 56 ± 9           |
| RY271 (ald7::HIS3)      | 100 ± 5  | 74 ± 5    | 5 ± 1    | 5 ± 2  | 40 ± 5           |

Effect of carbon source on pyruvate dehydrogenase complex activity and on kinetic parameters of pyruvate oxidation by isolated mitochondria from PS194 strain

Respiratory rates by isolated mitochondria given below were measured in the presence of 2 mM ADP and 5 mM DL-malate. The dehydrogenase activities were measured as reported under “Experimental Procedures” and in the presence of 0.1% Triton X-100. Experimental results are the average ± S.D. of measurements with at least three independent mitochondrial isolations.

| Carbon source for growth | Kinetic parameters of respiration on pyruvate (mV) | Specific activity (nmol/min/mg protein) |
|-------------------------|---------------------------------|--------------------------------------|
|                         | Kitt (nmol/min/mg protein) | Vmax (nmol/min/mg protein) | Pyruvate dehydrogenase | Acetaldehyde dehydrogenase |
| Yeast peptone lactate   | 0.38 ± 0.13          | 90 ± 15                           | 39 ± 8                    | 400 ± 35            |
| Yeast peptone glycerol  | 0.43 ± 0.15          | 70 ± 7                            | 30 ± 7                    | 450 ± 55            |
| Yeast peptone ethanol   | 0.46 ± 0.19          | 60 ± 4                            | 33 ± 5                    | 475 ± 59            |

In yeast cells, two kinds of acetaldehyde dehydrogenase have been characterized; one is Mg2+-activated (Mg2+-acetaldehyde dehydrogenase), NADP+–linked, and localized in the cytosol (8, 9), and the other is K+-activated (K+-acetaldehyde dehydrogenase), NAD(P)–linked, and localized in the mitochondrial matrix (25, 26). The former is thought to be involved in cytosolic biosynthetic pathways (26). Recently, ALD7 coding for the mitochondrial enzyme was identified and interrupted in the strain RY271 by the HIS3 gene (31). We verified that the mitochondrial K+-acetaldehyde dehydrogenase was lacking in the mutant by measuring its activity in cells grown under different conditions. The growth of mutant ald7Δ on glucose or lactate was not affected, whereas growth on ethanol was severely impaired (Table III).

In conclusion, mutants lacking either pyruvate dehydrogenase or mitochondrial acetaldehyde dehydrogenase activity grow on lactate. However, the co-disruption of both PDA1 and ALD7 genes abolishes the growth on lactate (Table III), showing that either K+- acetaldehyde dehydrogenase or pyruvate dehydrogenase is necessary for growth.

Oxygen Uptake Studies with Permeabilized Spheroplasts from Wild-type S. cerevisiae and Deletion Mutants—We compared the respiratory rate on pyruvate of permeabilized spheroplasts from the different mutants used above with their parental isogenic strain. The respiratory rate of the pdc1Δ pdc5Δ double mutant was very low on pyruvate compared with that in the parental strain (Table IV); this respiratory rate was stimulated by malate, indicating that the unique pathway of pyruvate oxidation in the mutant is the pyruvate dehydrogenase tricarboxylic cycle pathway. In contrast, spheroplasts from the strain T2–3C lacking pyruvate dehydrogenase activity oxidized pyruvate, but the respiration was not stimulated by malate. Table IV shows that ald7Δ strain did not oxidize pyruvate unless malate was added, pointing to an essential role of the mitochondrial K+-acetaldehyde dehydrogenase in the pyruvate oxidation process. The cytosolic pyruvate dehydrogenase bypass cannot replace the mitochondrial one, since we observed that the addition of the different cofactors (NADP+, CoASH, and ATP) did not restore the respiratory rate on pyruvate.

Taken together, these results indicate that the mitochondrial
K⁺-acetalddehyde dehydrogenase pathway could be an alternative to pyruvate oxidation by the pyruvate dehydrogenase complex, as proposed in Fig. 3.

**Effect of Carbon Source on Pyruvate Dehydrogenase Complex Activity and on Kinetic Parameters of Pyruvate Oxidation by Isolated Mitochondria at a Fixed α-malate Concentration**—
Most of the experiments described in this paper were done with cells grown on lactate, a substrate that is a direct metabolic precursor of pyruvate. To establish whether growth under our conditions influenced the level of pyruvate dehydrogenase and mitochondrial acetalddehyde dehydrogenase, we determined their activities on cells grown on three nonfermentable carbon source cultures: 2% lactate, 2% glycerol, and 2% ethanol. The pyruvate dehydrogenase activity in crude cell extract interplayed with the isolated decarboxylase, a cytosolic enzyme. Therefore, we measured these activities on mitochondrial extract, and we did not observe a significant difference regardless of the carbon source used (Table V). The respiration of isolated mitochondria of yeast cells cultured under different conditions was measured as a function of pyruvate concentration in the presence of a fixed malate concentration. The results show that the affinity of mitochondria for pyruvate was approximately the same whatever the culture condition (Table V). The apparent affinity constant was about 0.4 mM, which is near the Km value reported for the isolated pyruvate dehydrogenase complex (6) and that for pyruvate transport by mitochondria (5). These results show that malate-dependent pyruvate oxidation by mitochondria is not influenced by the carbon source (Table V). It should be noted that the respiratory rate on pyruvate is about 2-fold higher than the pyruvate dehydrogenase activity, suggesting the contribution of other dehydrogenases of tricarboxylic acid cycle to respiration. Similarly, the level of activity of the mitochondrial acetalddehyde dehydrogenase did not depend on the nature of the nonfermentable carbon source. The comparison of these activities indicated that the mitochondrial acetalddehyde dehydrogenase was approximately 10-fold higher than pyruvate dehydrogenase and similar to the isolated mitochondrial respiration on pyruvate, in the presence of the large excess of commercial pyruvate decarboxylase added in the medium (see above).

**DISCUSSION**

The present results show that pyruvate can be oxidized inside mitochondria by a pathway involving the mitochondrial acetalddehyde dehydrogenase. We propose to call this pathway the mitochondrial pyruvate dehydrogenase bypass to distinguish it from the cytosolic one described by Pronk et al. (15) (Fig. 3). This system can compensate the absence of pyruvate dehydrogenase when yeast grow aerobically on glucose or lactate (two pyruvate-donating substrates) because of the production of both intramitochondrial NADH and acetate. Up to now, no acetly-CoA synthetase in the mitochondrial matrix has been described. However, acetate can diffuse through the inner membrane, and acetly-CoA formed in the cytosol may enter the mitochondria via the carnitine system to fuel the tricarboxylic acid cycle (12), yet the pyruvate mitochondrial bypass does not seem to be essential when pyruvate dehydrogenase is functional. Recently, Tessier et al. (31) reported that deletion of **ADH3** renders the yeast unable to grow on ethanol. It has previously been reported that deletion of the gene encoding the mitochondrial alcohol dehydrogenase (**ADH3**) does not prevent growth on ethanol, suggesting a cytosolic metabolism for that alcohol under these conditions (32). Taken together, these reports suggest that the mitochondrial acetalddehyde dehydrogenase is necessary for growth on ethanol despite a lack of mitochondrial alcohol dehydrogenase. We can postulate that growth on ethanol required intramitochondrial oxidative metabolism, although the cytosolic NADH is oxidized by the NADH-dehydrogenase at the cytosolic side of inner membrane (33, 34). When **ADH3** is deleted, the acetalddehyde produced in the cytosol by alcohol dehydrogenase is oxidized, at least partly, in the mitochondria by acetalddehyde dehydrogenase. Since the oxidation of acetalddehyde to acetate by the NAD⁺-linked acetalddehyde dehydrogenase is a very exergonic reaction, this pathway should be sufficiently active to allow a growth on ethanol. On the contrary, growth of the **ald7** mutant on ethanol was poor, since acetalddehyde accumulation limits the mitochondrial ethanol oxidation. Moreover, it has already been shown that both cytosolic and mitochondrial acetalddehyde dehydrogenases are required for growth on ethanol, with neither being able to suppress the loss of the other (31). In conclusion, it appears that if the cytosolic acetalddehyde dehydrogenase is necessary for biosynthesis (15), the mitochondrial one is required for the bioenergetic pathway for growth on ethanol.

**Acknowledgments**—We thank Dr. P. Meaden for the gift of the RY601 and RY271 strains, Dr. H. Y. Steensma for T2–3D and T2–3C strains, Dr. S. Hofmann for YSH6,56–3D and YSH14,136–2D strains, and Dr. R. Cooke for contributions in editing the manuscript.

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