The *Saccharomyces cerevisiae* NDE1 and NDE2 Genes Encode Separate Mitochondrial NADH Dehydrogenases Catalyzing the Oxidation of Cytosolic NADH*

(Received for publication, May 13, 1998, and in revised form, July 17, 1998)

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*This work is part of the Delft University DIOC-6 program “Mastering the Molecules of Manufacturing” and of the project “From Gene to Product in Yeast, a Quantitative Approach,” which is subsidized by the European Community (EC Framework IV Cell Factory Program). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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During dissimilation of sugars via respiration by eukaryotic cells, glycolysis leads to NAD\(^+\) reduction in the cytosol, whereas mitochondrial oxidation of pyruvate via the pyruvate-dehydrogenase complex and the trichloroacetic acid cycle yields NADH in the mitochondrial matrix. As the mitochondrial inner membrane is impermeable to NADH (1, 2), respiratory growth requires continuous reoxidation of this cofactor in the cytosol as well as in the mitochondrial matrix.

The mitochondrial inner membrane of the yeast *Saccharomyces cerevisiae* contains at least two NADH:ubiquinone-6 oxidoreductases (‘NADH dehydrogenases’) that may couple the oxidation of NADH to the mitochondrial respiratory chain (2 - 5). The catalytic site of one of these, commonly referred to as the ‘internal’ NADH dehydrogenase, faces the mitochondrial matrix. Thus, it can oxidize the intramitochondrial NADH generated by the pyruvate-dehydrogenase complex and the TCA cycle (2). In contrast to the classical ‘complex I’ NADH dehydrogenases of higher eukaryotes, the ‘internal’ NADH dehydrogenase of growing *S. cerevisiae* cells is not proton translocating (5, 6). The enzyme consists of a single subunit encoded by the nuclear *NDE1* gene (7). Mutants in which *NDE1* is inactivated do not oxidize intramitochondrial NADH (8).

In addition to the *NDE1*-encoded ‘internal’ NADH dehydrogenase, *S. cerevisiae* is able to synthesize another inner membrane NADH dehydrogenase, commonly referred to as external NADH dehydrogenase, the catalytic site of which faces the intermembrane space (Refs. 2, 3, and 5; Fig. 1).

In contrast to the mitochondria of fungi and plants (9, 10), mammalian mitochondria do not harbor external NADH dehydrogenases and therefore depend on redox shuttle mechanisms to couple the oxidation of cytosolic NADH to internal NADH dehydrogenases (11). The presence of an external NADH dehydrogenase in yeast mitochondria correlates with the absence of a functional malate-aspartate shuttle (5, 12), one of the major redox shuttles in mammalian mitochondria (11). However, the key enzymes for two alternative systems, the glycerol-3-phosphate dehydrogenase system and the ethanol-acetaldehyde shuttle (Fig. 1), have both been demonstrated in *S. cerevisiae* (2, 13, 14). A recent study indicated that the glycerol-3-phosphate dehydrogenase system contributes to the oxidation of cytosolic NADH under certain conditions but that it is not essential for respiratory growth of *S. cerevisiae* (15). The relative importance of the various proposed systems for respiratory oxidation of cytosolic NADH by *S. cerevisiae* mitochondria is at present unclear.

Even under aerobic conditions, alcoholic fermentation rather than respiration is the predominant mode of sugar metabolism in *S. cerevisiae* (16). Fully respiratory growth on sugars is only possible during sugar-limited cultivation below the so called critical specific growth rate (*μ*\(_{\text{crit}}\)). Above *μ*\(_{\text{crit}}\), respiration and alcoholic fermentation occur simultaneously, even in sugar-limited cultures (17–19). Aerobic fermentation negatively affects the biomass yield on sugars (20). Therefore, biomass-directed industrial applications of *S. cerevisiae*, such as the production of bakers’ yeast and heterologous proteins, have to be performed at submaximal growth rates in aerobic, sugar-limited fed-batch cultures (21, 22). Competition between mitochondria and alcohol dehydrogenase for cytosolic NADH
Yeast Genes Encoding External NADH Dehydrogenase

Experimental Procedures

Yeast Strains and Maintenance—The S. cerevisiae strains used in this study are listed in Table I. They were grown to stationary phase in shake-flask cultures on a mineral medium with vitamins (23), which was set at pH 6.0 and contained 20 glt (24) gamma-dehydrogenase. After adding sterile glycerol (30% v/v), 2-ml aliquots were stored in sterile vials at −80 °C. These frozen stock cultures were used to inoculate precultures for batch shake-flask cultures on a mineral medium with vitamins (23) and remained above 50% of air saturation. The defined medium with vitamins was prepared as described by Verduyn and oxygen consumption, had remained constant (2% variation) for at least two volume changes. Steady-state cultures did not exhibit detectable metabolic oscillations. The pH was kept at 5.0 by an Applikon ADI 1030 biocell, via the automatic addition of 2% KOH. The fermenter was sparged with air at a flow rate of 0.5 liter·min⁻¹ using a Brooks 5876 mass-flow controller. The dissolved oxygen concentration was continuously monitored with an oxygen electrode (Ingold, model 34 100 3002) and remained above 50% of air saturation. The defined medium with vitamins was prepared as described by Verduyn et al. (23) and contained 7.5 glt 1 γ-glucose as the carbon source. Chemostat cultures were routinely checked for purity by phase-contrast microscopy and by plating on YPD agar plates.

Determination of Culture Dry Weight—Culture samples (10 ml) were filtered over preweighed nitrocellulose filters (pore size 0.45 μm, Gelman Sciences). After removal of medium, the filters were washed with demineralized water, dried in a Sharp type R-4700 microwave oven for 20 min at 360 W output, and weighed. Duplicate determinations varied by less than 1%.

Substrate and Metabolite Analyses—Glucose in reservoir media and supernatants of chemostat cultures was determined enzymatically with a hexokinase/glucose-6-phosphate dehydrogenase kit (Boehringer Mannheim). Concentrations of ethanol, glycerol, and acetate were determined by high pressure liquid chromatography (31). High pressure liquid chromatography analyses were confirmed by enzymatic analysis of these metabolites (32).

Gas Analysis—The exhaust gas of chemostat cultures was cooled in a condenser (2 °C) and dried with a Perma Pure dryer. Oxygen and carbon dioxide concentrations were determined with a Servomex type 1100A analyzer and a Beckman model 864 infrared detector, respectively. The exhaust gas flow rate was measured as described previously (33). Specific rates of carbon dioxide production and oxygen consumption were calculated as described by van Urk et al. (34).

Isolation of Mitochondria—Mitochondria were isolated from glucose-limited, aerobic chemostat cultures by a procedure based on that described for Candida utilis by Bruinenberg et al. (35). Biomass (approximately 1.5 g dry weight) was harvested by centrifugation (4 × 10⁵ g for 5 min) and resuspended in 30 ml of Tris-HCl buffer (0.1 M, pH 9.3) containing 10 mM Dithiothreitol and incubated at 30 °C for 10 min. After centrifugation (4 min, 1250 × g), the pellet was washed with 40 ml of buffer A (25 mM potassium phosphate, 1 mM MgCl₂, 1 mM EDTA, pH 7.5), containing 2% sorbitol and resuspended in 35 ml of buffer B containing 2 M sorbitol. 10.2 mg of zymolase (from Arthrobacter luteus, 20,000 units⁻¹, ICN Biochemicals) was dissolved in

![Diagram](image)

**FIG. 1.** Proposed mechanisms for respiratory oxidation of cytosolic NADH in *S. cerevisiae* (2, 5): schematic representation of compartmentation and electron flow. **Numbers** indicate the following enzymes: 1, cytosolic NAD⁺-dependent alcohol dehydrogenase; 2, mitochondrial NAD⁺-dependent alcohol dehydrogenase; 3, mitochondrial internal NADH dehydrogenase; 4, mitochondrial external NADH dehydrogenase; 5, cytosolic NAD⁺-dependent glycerol-3-phosphate dehydrogenase; 6, mitochondrial flavoprotein glycerol-3-phosphate dehydrogenase.

1 The abbreviations used are: SFH, short flanking homology; PCR, polymerase chain reaction.
in 0.1 ml of buffer A containing 2 M sorbitol and added to the cell suspension, which was subsequently incubated at 30 °C for 1 h. This incubation period was chosen based on control experiments in which lysis of spheroplasts was periodically assayed by dilution of samples in demineralized water (35). All subsequent steps were carried out on ice. After centrifugation (7 min, 2800 \( g \)), the sediment (pellet) was washed with 40 ml of buffer A containing 2 M sorbitol and resuspended in 10 ml of the same buffer. Subsequently, 30 ml of buffer A containing 0.2 M sorbitol was added dropwise while the suspension was slowly stirred with a magnetic stirrer bar. The spheroplast suspension was subjected to 10 strokes in a cooled Potter-Elvehjem homogenizer (100 rpm, clearance 28 \( \mu \mbox{m} \)). After centrifugation (10 min, 2000 \( g \)), the homogenate (supernatant) was separated from unbroken cells and debris and spun again (10 min, 7800 \( g \)). The resulting pellet, containing the mitochondria, was resuspended in 10 ml of buffer A containing 2 M sorbitol and resuspended in 20 ml of the same buffer. Mitochondrial preparations were estimated by the Lowry method. Oxygen uptake rates were calculated based on a dissolved oxygen concentration of 236 \( \mu \mbox{M} \) in air-saturated water at 30 °C. Respiratory control values were determined by adding 0.25 mM ADP (37). Oxygen uptake assays by addition of 5 mM glucose, 0.2 mM NADH, and L-glycerol-3-phosphate (5 mM), or ethanol (36) were carried out on ice. Therefore, NADH was generated in the presence of ethanol contamination, the possibility was investigated to generate NADH in the oxygen uptake assays by addition of glucose and NAD\(^+\), together with NAD\(^+\)-dependent glucose dehydrogenase from Bacillus megaterium (see “Experimental Procedures”). Oxygen uptake by wild-type mitochondria could be observed only when all three components of this system for NADH regeneration were added (data not shown). Using this system, the respiratory control ratio for oxidation of NADH by wild-type mitochondria was about 3 (Table III), indicating that NADH oxidation was functionally coupled to oxidative phosphorylation. The glucose dehydrogenase system was used in all further experiments on NADH oxidation by mitochondrial preparations.

**YMR145c and YDL085w Are Candidate Structural Genes for External NADH Dehydrogenase**—To identify candidate structural genes encoding the mitochondrial external NADH dehydrogenase of S. cerevisiae, the deduced amino acid sequence encoded by the unique gene encoding the internal NADH dehydrogenase of S. cerevisiae was compared with the entire S. cerevisiae genome sequence (39). Data base searches using services offered by MIPS (BLAST, 40) yielded two open reading frames with unknown function, YMR145c and YDL085w. Sequence alignment of Ndi1 to the predicted peptide sequences encoded by YMR145c and YDL085w revealed identities of 48% and 46%, respectively. An even higher identity of 63% was found when the predicted peptide sequence encoded by YMR145c and YDL085w were compared. The sequence identity was found along the whole length of the three predicted peptide sequences. Interestingly in contrast to Ndi1p, the putative peptides encoded by YMR145c and YDL085w showed N-terminal extensions of 30 and 45 amino acids, respectively. These extensions are of interest as they might theoretically be involved in targeting of the proteins to the appropriate subcellular organelles.
lar locations. YMR145c exhibits a codon adaptation index of 0.26, which is indicative of a moderately expressed gene, similar to NDII (codon adaptation index 0.19). YDL085w exhibited a lower codon adaptation index of 0.14. Based on their similarity with NDII, YMR145c and YDL085w were tentatively named NDE1 and NDE2 (NADH dehydrogenase, external), respectively.

Oxygen Uptake Studies with Mitochondria from Wild-type S. cerevisiae and Deletion Mutants—To investigate whether open reading frames YMR145c/NDE1 and YDL085w/NDE2 are indeed structural genes encoding mitochondrial external NADH dehydrogenases, NADH-dependent respiration was studied in isolated mitochondria from wild-type S. cerevisiae and from isogenic mutants in which either NDE1, NDE2, or both had been deleted.

Mitochondria were isolated from aerobic, glucose-limited chemostat cultures grown at a dilution rate of 0.10 h⁻¹. Under these conditions, wild-type S. cerevisiae did not exhibit alcoholic fermentation (see below), indicating that cytosolic NADH was efficiently reoxidized by the mitochondria. Such a situation cannot be achieved in batch cultures on glucose where, because of glucose repression of respiratory enzymes, alcoholic fermentation is the predominant mode of NADH reoxidation (16, 41).

As discussed above, wild-type mitochondria readily oxidized exogenous NADH. The respiratory control ratio of 3 (Table III) strongly suggested that this NADH-oxidizing activity was due to the presence of an external NADH dehydrogenase rather than to exposure of the internal enzyme because of the presence of damaged mitochondria. Wild-type mitochondria were also capable of oxidizing pyruvate when this substrate was added in combination with malate. This indicates that the oxidation of intramitochondrial NADH, formed by the pyruvate-dehydrogenase complex and the trichloroacetic acid cycle, could be functionally coupled to the respiratory chain via internal NADH dehydrogenase. Similarly, the respiration rates observed with l-glycerol-3-phosphate, succinate, and ethanol (Table III) were indicative of functional coupling of mitochondrial glycerol-3-phosphate dehydrogenase, succinate dehydrogenase, and mitochondrial alcohol dehydrogenase to the respiratory chain.

Deletion of NDE1 caused a 3–4-fold decrease in NADH-dependent oxygen uptake by mitochondria, whereas no decrease was observed for the other substrates tested (Table III, Fig. 2). The residual NADH-oxidizing activity in mitochondrial preparations of the mutant still exhibited respiratory control (Table III), indicating that this activity was not entirely due to contamination with nonrespiratory chain-linked oxidases (42).

Deletion of NDE2 alone did not have a significant effect on the rate of NADH oxidation by isolated mitochondria, nor was there a clear effect observed for any of the other substrates tested (Table III, Fig. 2). However, when both NDE1 and NDE2 were deleted, NADH-dependent oxygen uptake by isolated mitochondria was completely abolished (Table III, Fig. 2). In the nde1Δ nde2Δ mutant, oxidation rates with other substrates were not significantly lower than in the wild type (Table III, Fig. 2), indicating that the nde deletions did not affect coupling of other dehydrogenase systems to the respiratory chain.

Growth Characteristics of nde Null Mutants in Chemostat Cultures—In aerobic, glucose-limited chemostat cultures of the wild-type strain CEN.PK113–7D grown at a dilution rate of 0.10 h⁻¹, neither ethanol nor glycerol was found in culture supernatants, and all glucose carbon in the feed could be quantitatively recovered as biomass and carbon dioxide (Table IV). The biomass yield on glucose was 0.49 g of biomass/g glucose⁻¹, which is typical for respiratory growth of wild-type S. cerevisiae strains (20). A further confirmation that glucose metabolism in these cultures was fully respiratory was that the ratio between specific rates of carbon dioxide production and oxygen uptake was close to unity (Table IV).

Also in the nde deletion mutants, growth was essentially respiratory, as evident from the absence of ethanol in culture supernatants and a respiratory coefficient close to 1 (Table IV). Both in the nde1Δ mutant and in the nde1Δ nde2Δ double mutant, low concentrations of glycerol were detected in culture supernatants (Table IV). Glycerol formation is the major pathway for reoxidation of cytosolic NADH during anaerobic growth.

### Table III

| Substrate | S. cerevisiae CEN.PK113–7D | S. cerevisiae CEN.PK152 (nde1Δ nde2Δ) | S. cerevisiae CEN.PK162 (nde1 nde2Δ) | S. cerevisiae CEN.PK167–2B (nde1Δ nde2Δ) |
|-----------|----------------|---------------------------------|-----------------------------------|-----------------------------------|
|           | O₂ uptake rate | RC | O₂ uptake rate | RC | O₂ uptake rate | RC | O₂ uptake rate | RC |
| NADH      | 0.22 ± 0.06    | 3.0 ± 0.3 | 0.08 ± 0.04    | 1.8 ± 0.2 | 0.28 ± 0.01    | 2.6 ± 0.1 | 0.0 ± 0.0      | 1.0 ± 0.0 |
| Malate + pyruvate | 0.11 ± 0.01    | 1.7 ± 0.1 | 0.16 ± 0.01    | 2.0 ± 0.3 | 0.15 ± 0.01    | 2.0 ± 0.2 | 0.15 ± 0.02    | 2.0 ± 0.1 |
| Ethanol   | 0.10 ± 0.02    | 1.4 ± 0.1 | 0.11 ± 0.02    | 1.4 ± 0.1 | 0.16 ± 0.03    | 1.7 ± 0.1 | 0.15 ± 0.03    | 1.6 ± 0.1 |
| l-glycerol-3-phosphate | 0.18 ± 0.04 | 2.3 ± 0.1 | 0.22 ± 0.06 | 2.5 ± 0.3 | 0.25 ± 0.04 | 2.2 ± 0.3 | 0.26 ± 0.03 | 2.6 ± 0.3 |
| Succinate | 0.10 ± 0.02    | 1.6 ± 0.1 | 0.13 ± 0.01    | 2.4 ± 0.4 | 0.13 ± 0.02    | 1.9 ± 0.2 | 0.17 ± 0.04    | 3.0 ± 0.3 |
of *S. cerevisiae* (14, 43). However, the amount of glycerol produced by the mutant strains corresponded to less than 1% of the glucose carbon fed to the cultures, indicating that glycerol production was not a major means of reoxidizing cytosolic NADH in these aerobic cultures (Table IV).

The biomass yield of the nde1Δ nde2Δ strain in the glucose-limited chemostat cultures was about 10% lower than that of the wild type. As this difference cannot be explained from the small amounts of glycerol produced by the cultures, it suggests that rerouting of the oxidation of cytosolic NADH via alternative pathways led to a lower energetic efficiency.

Growth of Wild-type *S. cerevisiae* and nde Mutants in Shake-flask Cultures—To further investigate the phenotype of nde mutants, specific growth rates were determined in shake-flask cultures. In glucose-grown cultures, deletion of NDE1, NDE2, or both had no significant effect on the specific growth rate (Table V). This is consistent with the notion that alcoholic fermentation rather than respiration is the key mode of glucose dissimilation in batch cultures (16).

Galactose represses respiratory enzymes to a lesser extent than glucose and is metabolized at a lower rate than glucose by wild-type *S. cerevisiae* strains. Consequently, during batch cultivation on galactose, the relative contribution of respiration is larger than during growth on glucose (16). The specific growth rates of the nde1Δ and nde1Δ nde2Δ mutants on galactose were about 30% lower than those of the isogenic wild type. Deletion of only NDE2 did not have a significant effect on the specific growth rate on galactose (Table V). This indicates that Nde1p is involved in the dissimilation of galactose via respiration and that alternative systems for reoxidation of cytosolic NADH cannot sustain wild-type growth rates.

*S. cerevisiae* contains both mitochondrial and cytosolic isoenzymes of alcohol dehydrogenase and acetaldehyde dehydrogenase (2, 44). Exclusive involvement of the mitochondrial isoenzymes of these dehydrogenases might, at least in theory, prevent the generation of cytosolic NADH in the initial steps in ethanol metabolism. Nevertheless, deletion of both NDE1 and NDE2 caused a significant reduction of the specific growth rate on ethanol. The corresponding single mutants exhibited essentially the same specific growth rate on ethanol as the wild-type (Table V).

**DISCUSSION**

In this study, the *S. cerevisiae* open reading frames YMR145c/NDE1 and YDL085w/NDE2 have been unambiguously identified as two structural genes that each encode a mitochondrial external NADH dehydrogenase. An independent corroboration of the identity of NDE1 was obtained by sequencing of the N-terminal amino acid sequence of a NADH dehydrogenase purified from an *S. cerevisiae* mutant lacking the

### Table IV

|                          | S. cerevisiae CEN.PK113–7D (NDE1 NDE2) | S. cerevisiae CEN.PK152 (nde1Δ NDE2) | S. cerevisiae CEN.PK152 (NDE1 nde2Δ) | S. cerevisiae CEN.PK167–2B (nde1Δ nde2Δ) |
|-------------------------|----------------------------------------|------------------------------------|-------------------------------------|------------------------------------------|
| Biomass yield (g biomass  \( \cdot \) g glucose  \(^{-1}\)) | 0.49 ± 0.00                           | 0.48 ± 0.01                        | 0.49 ± 0.01                         | 0.45 ± 0.01                             |
| Residual glucose (mM)   | <0.2                                   | <0.2                              | <0.2                                | <0.2                                    |
| Ethanol (mM)            | <0.1                                   | <0.1                              | <0.1                                | <0.1                                    |
| Glycerol (mM)           | <0.1                                   | 0.57 ± 0.03                        | <0.1                                | 0.78 ± 0.03                             |
| qCO\(_2\) (mmol  \( \cdot \) g  \(^{-1}\)  \( \cdot \) h  \(^{-1}\)) | 2.7 ± 0.1                             | 2.9 ± 0.1                         | 2.7 ± 0.1                           | 3.0 ± 0.1                               |
| qO\(_2\) (mmol  \( \cdot \) g  \(^{-1}\)  \( \cdot \) h  \(^{-1}\)) | 2.6 ± 0.1                             | 2.9 ± 0.1                         | 2.6 ± 0.1                           | 3.0 ± 0.1                               |
| RQ                     | 1.03 ± 0.02                           | 0.99 ± 0.01                        | 1.03 ± 0.02                         | 1.00 ± 0.01                             |
| Carbon recovery (%)     | 98 ± 1                                 | 100 ± 1                           |                                     |                                         |

### Table V

| Strain and relevant genotype | Specific growth rate (h  \(^{-1}\)) |
|-----------------------------|----------------------------------|
|                             | Glucose | Galactose | Ethanol |
| S. cerevisiae CEN.PK113–7D (NDE1 NDE2) | 0.40    | 0.18      | 0.17    |
| S. cerevisiae CEN.PK152 (nde1Δ NDE2) | 0.38    | 0.12      | 0.15    |
| S. cerevisiae CEN.PK162 (NDE1 nde2Δ) | 0.39    | 0.17      | 0.17    |
| S. cerevisiae CEN.PK167–2B (nde1Δ nde2Δ) | 0.37    | 0.12      | 0.12    |

*NDH1*-encoded internal NADH dehydrogenase. The obtained sequence of the purified 54-kDa flavoprotein was XXXX-VILQRKAVT (i.e. the first four amino acids could not be identified; the T was ambiguous). The residues VILQR correspond to amino acids 46–52 of the predicted amino acid sequence of Nde1p.

Identification of NDE1 and NDE2 required a special protocol for generation of ethanol-free NADH in the oxygen-uptake experiments with isolated mitochondria. The importance of this experimental detail became evident when commercial NADH was used in preliminary studies with mitochondria isolated from the nde1Δ nde2Δ mutant. In these studies, a significant residual respiration rate was observed (0.05 mmol O\(_2\)  \( \cdot \) min  \(^{-1}\)  \( \cdot \) mg protein  \(^{-1}\)). Control experiments in which ethanol was provided at the same concentration as was calculated to be present in the experiments with commercial NADH yielded the same (<10% difference) respiration rates as those observed with commercial NADH. The indirect method for generating NADH used in this study may also be applicable in other systems in which ethanol interferes with the quantitation of NADH-dependent reactions.

Although mitochondria from the nde1Δ nde2Δ mutant failed to oxidize external NADH (Table III), glucose-limited, aerobic chemostat cultures grown at a dilution rate of 0.10 h  \(^{-1}\) did not exhibit alcoholic fermentation. Instead, glucose dissimilation occurred virtually completely via respiration (Table IV). This observation constitutes the first experimental evidence that systems other than the external NADH dehydrogenase can sustain mitochondrial reoxidation of cytosolic NADH in growing *S. cerevisiae*. As indicated in the literature (2, 5, 15), both the glycerol-3-phosphate system and an ethanol-acetaldehyde shuttle might theoretically fulfill this role. The small amounts

2 S. de Vries, unpublished data.
of glycerol produced by glucose-limited cultures of the nde1Δ nde2Δ mutant (Table IV) may be indicative of an increased activity of the glycerol-3-phosphate dehydrogenase system compared with the wild type. Although glycerol itself is not an intermediate in this proposed system for NADH reoxidation (Fig. 1), it can be formed by dephosphorylation of glycerol-3-phosphate via the GPP1 and GPP2-encoded glycerol-3-phosphates (15, 45).

With the identification of the NDE1 and NDE2 genes, structural genes have now been identified for all three major mechanisms proposed to contribute to the reoxidation of cytosolic NADH by S. cerevisiae mitochondria (Fig. 1). Construction of mutants in which different combinations of these proposed systems have been eliminated will eventually show whether all three systems can function in growing S. cerevisiae and whether other redox shuttle systems are also operating.

Under the experimental conditions investigated in this study, the phenotype of null mutants seems to indicate that Nde1p is the more important of the two external NADH dehydrogenases; absence of Nde2p did not, by itself, result in a clear phenotype. Of course, the relative expression of NDE1 and NDE2 may strongly depend on growth conditions. A recent study on transcription of the yeast genome has demonstrated that both YMR145c/NDE1 and YDL085w/NDE2 are transcribed during growth on glucose in batch cultures, with NDE2 transcription being strongly induced when the cultures switched to ethanol utilization (46). Further studies, involving a broad range of growth conditions, are required to investigate the regulation of these two external NADH dehydrogenases.

Identification of the NDE1 and NDE2 genes makes S. cerevisiae the first eukaryote in which the genes encoding external NADH dehydrogenase have been identified. It will be of interest to investigate whether, and to what extent, Nde1p and Nde2p are similar in structure, function, and regulation to the external NADH dehydrogenases in other fungi and in plants. Functional complementation of S. cerevisiae nde1Δ nde2Δ mutants with plant homologues may prove to be an attractive model system for such studies.

Acknowledgment—We thank Prof. Dr. K-D. Entian for stimulating discussions and support.

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