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Alternative Type II NAD(P)H Dehydrogenases in the Mitochondria of Protists and Fungi

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Plants, fungi, and some protists possess a more branched electron transport chain in their mitochondria compared to canonical one. In these organisms, the electron transport chain contains several rotenone-insensitive NAD(P)H dehydrogenases. Some are located on the outer surface, and others are located on the inner surface of the inner mitochondrial membrane. The putative role of these enzymes still remains elusive, but they may prevent the overreduction of the electron transport chain components and decrease the production of reaction oxygen species as a consequence. The last two decades resulted in the discovery of alternative rotenone-insensitive NAD(P)H dehydrogenases present in representatives of fungi and protozoa. The aim of this review is to gather and focus on current information concerning molecular and functional properties, regulation, and the physiological role of fungal and protozoan alternative NAD(P)H dehydrogenases.

Key words: Protists; fungi; rotenone-insensitive NAD(P)H dehydrogenases; mitochondrial electron transport chain; energy metabolism.

Contents

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Introduction

The mitochondria of eukaryotes constitute a platform for a variety of processes that provide cell energy homeostasis. They control energy production and dissipation to maintain the proper conditions for the cell to function. In a canonical respiratory chain, there are four basic protein complexes enabling electron transport and proton pumping that result in electrochemical gradient generation and ATP synthesis. In contrast to the classical constitution of the mammalian respiratory chain, plants, fungi, and protists contain additional components that branch from the primary canonical chain (Moller 2001; Rasmusson et al. 2008). These extra components include rotenone-insensitive type II NAD(P)H dehydrogenases or alternative NAD(P)H dehydrogenases (NDH2) and a cyanide-insensitive alternative oxidase (AOX), which all belong to mitochondrial energy-dissipating systems (Fig. 1). They dissipate energy indirectly, since they do not pump protons and provide a bypass of electrons from the classical respiratory complexes. In this manner, type II NAD(P)H dehydrogenases and the AOX modulate the efficiency of energy conservation by the mitochondrial respiratory chain and ATP synthesis through oxidative phosphorylation (Moller 2001; Rasmusson et al. 2008). Interestingly, recent studies have identified several *Arabidopsis thaliana* type II NAD(P)H dehydrogenases as dual targeted proteins (Xu et al. 2013). They target either mitochondria and peroxisomes or mitochondria and chloroplasts. The dual targeting ability of NDH2 probably arose early in the evolution of land plants. However, the NDH2 are usually described in relation to the role they play in the mitochondria.

General Characteristics of the Type II Dehydrogenases

Alternative NAD(P)H dehydrogenases conduct the reaction of the rotenone-insensitive oxidation of cytosolic and mitochondrial matrix NADH and/or NADPH (Fig. 1) (Melo et al. 2004; Rasmusson et al. 2004, 2008). They catalyze the two-step transfer of electrons to ubiquinone (UQ), providing the bypass of the rotenone-sensitive Complex I (NADH:UQ oxidoreductase, NADH dehydrogenase, or NDH1) when the matrix NAD(P)H is oxidized or the alternative electron path to Complex I when the cytosolic NAD(P)H is oxidized. The NDH2 are small proteins that are tens of kDa (usually 50–60 kDa) generally anchored by the C-terminus in the inner mitochondrial membrane and located towards either the matrix (internal dehydrogenases, NDI) or the intermembrane space (external dehydrogenases, NDE) (Melo et al. 2004; Rasmusson et al. 2004, 2008). The manner in which NAD(P)H dehydrogenases are embedded in the membrane remains unclear. There are several reports showing that a hydrophobic environment is necessary for these enzymes to be active (Bandeiras et al. 2003; Bjorklof et al. 2000; Gomes et al. 2001). Some in silico predictions of the secondary structures of type II dehydrogenases revealed putative transmembrane α-helices, e.g., *Neurospora crassa* NDE1 and *Saccharomyces cerevisiae* NDI1 (Melo et al. 2004). On the other hand, it has been suggested that the NDH2 interact with the membrane with the aid of amphipathic α-helices, where the hydrophobic and hydrophilic residues are situated on opposite sides on the helical surface (Bandeiras et al. 2002; Melo et al. 2004). In spite of this, the mechanism of the precise tethering of the protein to the membrane is unknown, and further structural studies are necessary to clarify the issue.

Type II dehydrogenases are usually monomers or homodimers. Their amino acid sequences contain two well conserved motifs to bind nucleotides, one is designated for the noncovalent binding of FAD or FMN, while the second is for NAD(P)H (Kerscher 2000; Melo et al. 2004; Rasmusson et al. 2004). The NAD(P)H dehydrogenases possess a type I UQ-binding site, whose characteristic feature is one conserved histidine residue flanked downstream by an aliphatic residue (usually leucine) (Fisher and Rich 2000). In addition, the UQ-binding sites differ somewhat between the NDH2 originating from various organisms, probably indicating the adaptation to the UQ molecule present in a particular organism, and thus enabling a more effective protein-UQ interaction (Melo et al. 2004). Alternative NAD(P)H dehydrogenases are classified into three groups depending on the conserved motifs present in their primary sequences and secondary structures. Group A includes the NDH2 with two ADP-binding motifs engaged in the noncovalent binding of NAD(P)H and flavin nucleotides, while group B comprises the NDH2 that possess both ADP-binding motifs and a conserved EF-hand fold that is responsible for calcium binding (Bandeiras et al. 2003; Gomes et al. 2001). The dehydrogenases from both groups are found in eukaryotes, archaea, and bacteria. Group C contains enzymes with a conserved consensus motif in a $\beta\alpha\beta$ fold and with a covalently bound flavin nucleotide. This group has previously been limited to hyperthermophilic archaea, but cyanobacterial proteins and

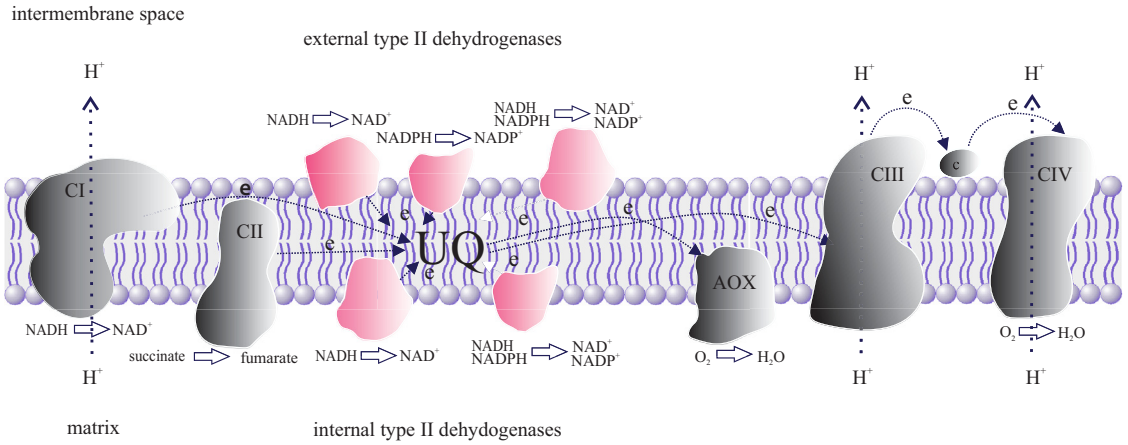

Figure 1. Schematic presentation of the branched mitochondrial respiratory chain with the location of type II dehydrogenases. Complex I (CI) is absent in *S. cerevisiae*, *K. lactis*, *Plasmodium* and *Toxoplasma*; AOX (alternative oxidase) is absent in *S. cerevisiae*, *K. lactis*, and *P. yoelli*. In fungal mitochondria, internal alternative dehydrogenases usually use NADH as a substrate; in *T. brucei* and *P. falciparum*, they can oxidize both NADH and NADPH. In fungi and protists, external alternative dehydrogenases oxidize NADH or NADH and NADPH. CI-IV, respiratory complexes I–IV.

A. thaliana NDC1 and rice NDC homolog have been classified in this group (Michalecka et al. 2003). NDC1 has been found in proteomic analyses in plastoglobules, indicating that it may be present in both mitochondria and chloroplasts (Ytterberg et al. 2006). Thus, the *ndc1* gene has been proposed to have entered the plant cell through the chloroplast progenitor and later transferred to the nucleus (Michalecka et al. 2003). NDI and NDE dehydrogenases in plants are correlated to the *nda* and *ndb* gene types, respectively (Michalecka et al. 2003; Rasmusson et al. 1999). The plant NDA family is the most similar to a homolog in *Trypanosoma brucei*. The *Neurospora crassa* NDE1 contains a conserved segment corresponding to the plant NDB EF-hand domain that is responsible for calcium binding and/or regulation. However, it has been suggested that because of their relatively low level of similarity, these insertions may be descendants of a single evolutionary event, from which the N-terminal EF-hand motif is conserved in the NDB and the C-terminal in the *N. crassa* NDE1 (Kerscher 2000; Michalecka et al. 2003). Using molecular modeling, it has been recently demonstrated that the NDB sequences possess different motifs, some of which are the acidic-type, and the others are the non-acidic-type. Among the NDB proteins, the presence of non-acidic and acidic motifs correlates with specificity for NADPH and NADH, respectively (Hao and Rasmusson 2016).

The presence of external NAD(P)H dehydrogenases (NDE) on the outer surface of the inner mitochondrial membrane enables the mitochondria to oxidize cytosolic NAD(P)H (Moller 2001). A porin (a voltage-dependent anion-selective channel, VDAC) that is abundant in the outer mitochondrial membrane permits the passage of molecules of <5 kDa and thus ionically and metabolically connects the intermembrane space to the cytosol. A substantial amount of evidence, primarily in plants and fungi, points to the existence of two separate enzymes oxidizing either cytosolic NADH or cytosolic NADPH in the inner mitochondrial membrane (Carneiro et al. 2004, 2007; Fredlund et al. 1991; Luttik et al. 1998; Melo et al. 2001; Michalecka et al. 2004; Rigoulet et al. 2004; Roberts et al. 1995). Specifically, NADPH oxidation is more sensitive to inhibition by diphenyleneiodonium chloride (IDP) than NADH. However, because diphenyleneiodonium chloride is not sufficiently specific, it substantially complicates the research (Kerscher 2000; Rasmusson et al. 2004). Some alternative dehydrogenases contain a sequence with a more or less degenerate EF-hand motifs that indicates Ca^{2+} binding (Michalecka et al. 2003; Rasmusson and Moller 1991). In plant mitochondria, Ca^{2+} dependence has been observed for external NADH and NADPH oxidation (Moller and Palmer 1981; Moller et al. 1982; Rasmusson et al. 2004) with NADH oxidation being less sensitive

(Arron and Edwards 1980). Moreover, NADPH oxidation appears to require more Ca^{2+} for activity than NADH oxidation, which could strengthen the possibility that there are two separate enzymes for NADH and NADPH. Interestingly, in most cases, the NADH-oxidizing enzymes can also oxidize NADPH due to the structural similarity of both molecules that differ in the presence of a phosphate group in NADPH. However, in alkaline pH, the oxidation of NADPH could be prevented by electrostatic repulsion between the negative charges at the phosphate group of NADPH and the phospholipids of the membrane (Moller and Palmer 1981). There are also reports that demonstrate the dependence of the internal NAD(P)H dehydrogenases (NDI) on calcium ions (Rasmusson and Moller 1991). The calcium effect is thought to be mediated by an electrostatic screening of the negatively charged phospholipid membrane, which allows for the negatively charged NAD(P)H molecule to approach the membrane, thus increasing the affinity for NAD(P)H (Johnston et al. 1979). Because alternative dehydrogenases exhibit maximal activity in acidic pH values, this attribute seems to play a significant role in providing the proper activity of these enzymes at the physiological pH and/or under stress conditions. In addition, changes in the pH and Ca^{2+} may differentially regulate NAD(P)H oxidation and therefore affect the cellular redox state (Rasmusson et al. 2008). Recently, it has been proposed that EF-hand motifs may constitute sites for regulation of enzyme activity by acting on the residues that stabilize/protonate FAD in different oxidation states during enzymatic reaction (Marreiros et al. 2017).

The number, substrate specificity, and Ca^{2+} dependence of alternative NAD(P)H dehydrogenases vary significantly when different organisms are compared. For instance, *Arabidopsis* mitochondria may possess up to seven proteins, including three external dehydrogenases: two are NADH dehydrogenases, one of which is calcium-dependent the other is calcium-independent, and the third dehydrogenase is a Ca^{2+}-dependent NADPH enzyme (Geisler et al. 2007). The other three enzymes are confirmed to locate towards the matrix side, and one, NDC1, has also been found in plastoglobules (Ytterberg et al. 2006). When examining the sequence homology to type II NADH dehydrogenases in yeast and *E. coli*, only two genes, *nda1* and *ndb1*, were found in potato. Based on the results of the protein import into the mitochondria, the NDA1 and NDB1 proteins have been determined to be directed to the inner and outer surface of the inner mitochondrial membrane, respectively (Rasmusson et al. 1999).

The last decades have introduced many new data on the presence and features of alternative NAD(P)H dehydrogenases in fungi and protists. In this review, we present the current state of knowledge of fungal and protozoan NDH2, including molecular and functional properties, regulation, and physiological impact.

Type II NAD(P)H Dehydrogenases in Fungi

Fermentative Yeast

Saccharomyces cerevisiae

Because *S. cerevisiae* is a facultative anaerobic yeast capable of meeting its energy requirements with ATP generated by fermentation, only relatively few mitochondrial proteins are essential for cell viability. The mitochondrial respiratory chain of baker's yeast does not contain Complex I. Instead of Complex I, it possesses an internal alternative NADH dehydrogenase NDI1 that functions as a matrix NADH-oxidizing enzyme and thus maintains matrix NAD^+/NADH homeostasis (Rigoulet et al. 2004). Due to the lack of transhydrogenase activity in yeast, the enzyme is essential for the yeast cell to preserve the correct redox balance both in the mitochondrial matrix and in the cytosol. An analysis of the crystal structure indicates that the yeast NDI1 forms a globular α/β structure that can be divided into three domains: an active part with two domains, one for FAD and one for NADH binding, and a C-terminal domain (Feng et al. 2012; Iwata et al. 2012). The C-terminal domain is usually highly conserved among various species. It consists of three β-strands and two α-helices, and the α-helices form a helix-turn-helix structure that interacts extensively with the active domain of NDI1. In addition, C-terminal domain participates through helix α16 in homodimer formation of NDI1 of approximate size $\sim$150 kDa (Feng et al. 2012). Moreover, deletion analysis indicates that helices α15 and α16 are strongly involved in anchoring the protein to the membrane. Like many enzymes that react with UQ, NDI1 has two UQ-binding sites, UQ_I and UQ_{II}. UQ_I may not readily exchange with the UQ pool because it forms extensive interactions with the CTD of NDI1. However, in contrast, UQ_{II} is more likely to serve this purpose because it has much fewer contacts with NDI1. The structures obtained suggest that there is an electron transfer pathway from NADH to UQ_{II}, with UQ_I acting as an intermediate together with FAD. FAD first accepts two

electrons from NADH to form $FADH_2$ and subsequently transfers them to UQ_I and UQ_{II} (Feng et al. 2012). However, the exact mechanism of the electron transfer has not still been elucidated in detail. Recently, Yamashita et al. (2018) have reported that stigmatellin (STG) acts as a first known competitive inhibitor of NDI1. It has been hypothesized that the stigmatellin binding site overlaps with the UQ_I site during the enzymatic reaction. The identification of binding UQ as a substrate at the STG-1a/UQ_I site implies that the binding sites for two substrates, UQ and NADH, are distinct. According to the authors STG-1b/UQ_{II} may not be functional in the enzymatic reaction of NDI1 (Yamashita et al. 2018).

In the yeast mitochondria, external and internal alternative NADH dehydrogenases have been reported to form a large supercomplex with succinate dehydrogenase (Complex II) (Grandier-Vazeille et al. 2001). Alternatively, the yeast mitochondrial NDI1 has recently been described to associate with Complexes III and IV, forming a respirosome-like structure, which can facilitate electron channeling between individual respiratory complexes (Matus-Ortega et al. 2015). NDI1 has garnered extensive attention due to experiments using the enzyme to restore the mitochondrial electrochemical potential in *Caenorhabditis elegans* and mammalian cells with Complex I defects (DeCorby et al. 2007; Perales-Clemente et al. 2008) and opens the possibility of genetic treatment for Parkinson's and LEBER optic neuropathic diseases (Marella et al. 2007, 2008). In addition, cells transfected with the *ndi1* gene decreased the production of reactive oxygen species (ROS) (DeCorby et al. 2007; Marella et al. 2007; Seo et al. 2006).

Notably, the *S. cerevisiae* mitochondria do also possess external NADH dehydrogenases (Luttik et al. 1998). There are two enzymes present in the yeast inner mitochondrial membrane facing intermembrane space, NDE1 and NDE2. These dehydrogenases and glycerol-3-phosphate shuttle supply excess cytosolic NADH to the mitochondrial respiratory chain. In the null mutant *Δnde1Δnde2*, the ability to oxidize external NADH was abolished but did not result in lethality. This observation suggests that some other systems that sustain the mitochondrial reoxidation of cytosolic NADH must be present in the yeast mitochondria (Luttik et al. 1998). What is tremendously intriguing is that both NDE enzymes in yeast were confirmed to associate in a supramolecular complex with other dehydrogenases, thus likely influencing the activity of each other depending on substrate availability (Rigoulet et al. 2004). This finding indicates that in the yeast mitochondria, the metabolic pathway of cytosolic NADH is highly organized and regulated. For instance, activity of the external NADH dehydrogenases has an inhibitory effect on the external glycerol-3-phosphate dehydrogenase. The exact function of the external NADH dehydrogenases in yeast, apart from maintaining the NAD^+/NADH balance, still remains elusive. According to Fang and Beattie, external NADH dehydrogenases are responsible for half of the superoxide radicals produced in the yeast mitochondria during the transfer of electrons to oxygen (Fang and Beattie 2003a).

Kluveromyces lactis

Similar to *S. cerevisiae*, *K. lactis* is a facultative anaerobic respiratory yeast, which lacks Complex I in its mitochondrial respiratory chain. However, both yeasts differ regarding their respiratory-fermentative metabolism as *K. lactis* exhibits a greater use of the pentose phosphate pathway (PPP) to metabolize glucose instead of the fermentation process. Therefore, *K. lactis* respiration is not subject to glucose repression (Gonzalez-Siso et al. 2000). Based on the homology to the *S. cerevisiae* genes encoding alternative dehydrogenases, two genes, one for internal NADH dehydrogenase and one for external NADPH dehydrogenase, have been identified in the *K. lactis* genome and designated KlNDI1 and KlNDE1, respectively (Tarrio et al. 2005). An expression profile of the internal dehydrogenase KlNDI1 demonstrates flexibility dependent on the carbon source used by the cells. The level of the KlNDI1 transcript increases significantly when the cells grow in a low glucose concentration and in non-fermentable carbon sources. A similar effect has been observed in *S. cerevisiae* for its NDI1 protein that is more abundant in cells grown in non-fermentable carbon sources than in glucose. Importantly, the cloned KlNDI1 gene can complement the *ndi1* mutation of *S. cerevisiae* in vivo. In turn, KlNDE1 is external dehydrogenase that uses NADPH as a substrate. Its expression increases simultaneously with the activation of the oxidative branch of the pentose phosphate pathway. However, the absence of the downregulation of KlNDE1 at a high glucose concentration supports the Crabtree-negative phenotype of *K. lactis*. It has been proposed that the presence of the KlNDI1 and KlNDE1 dehydrogenases enables rapid adaptation to the different levels of carbon source and provides glucose metabolism via the pentose phosphate pathway at the expense of glycolysis (Tarrio et al. 2005).

Similar to *S. cerevisiae*, in *K. lactis* mitochondria, the second external dehydrogenase, KlNDE2, has

also been identified and characterized (Tarrio et al. 2006). In contrast to KlNDE1, the KlNDE2 protein uses both NADH and NADPH as substrates. A comparison of both enzymes shows that the Michaelis constant (K_M) of KlNDE2 for NADPH is almost twice as high as that of KlNDE1, indicating that KlNDE2 has a lower affinity of for NADPH. Thus, the cytosolic concentration of NADPH may impact the relative activity of each of these proteins. This fact suggests that *K. lactis* can modulate the synthesis of the two external alternative dehydrogenases in relation to the requirements of the cell. Surprisingly, in spite of the EF-like motif present in the KlNDE2 sequence, there is no evidence of calcium regulation of the enzyme (Tarrio et al. 2006).

Considering the ability to oxidize cytosolic NADPH in the mitochondria, both fermentative yeast described, *S. cerevisiae* and *K. lactis*, exhibit a substantially different physiology. In contrast to *S. cerevisiae*, *K. lactis*, which is highly dependent on glucose oxidation via the pentose phosphate pathway that produces NADPH, requires the oxidation of cytosolic NADPH in the mitochondria.

Filamentous Fungi

Neurospora crassa

Four alternative NAD(P)H dehydrogenases have been cloned and characterized in the mitochondria of the filamentous fungus *N. crassa* (Carneiro et al. 2004, 2007; Duarte et al. 2003; Melo et al. 2001). One of these enzymes is directed towards mitochondrial matrix (NDI1), while three others are directed externally (NDE1-3). However, one of them seems to be present in the cytosol as well. An NDI1 protein of ~57 kDa is located internally in the inner mitochondrial membrane, which was confirmed using digitonin fractionation and proteinase K treatment of the mitochondria (Duarte et al. 2003). Based on experiments on the activity of the isolated NDI1 protein, a K_M value of 56 μM for NADH was determined using Q2 as electron acceptor. In the presence of rotenone, the *ndi1* mutant almost completely lacked NADH oxidation ability, suggesting that NDI1 is the sole internal alternative dehydrogenase in the *N. crassa* mitochondria. Interestingly, NDI1 may play a key role in the initial steps of spore germination because its absence delays the process (Duarte et al. 2003).

Studies on the external dehydrogenases of *N. crassa* have shown that the NDE1 protein is a Ca^{2+}-dependent external NADPH dehydrogenase, while NDE2 is characterized as a dehydrogenase oxidizing both cytosolic substrates (external NADH and/or NADPH) (Carneiro et al. 2004; Melo et al. 2001). All three dehydrogenases of *N. crassa* (NDI1 and NDE1,2) are not essential proteins for the function of the fungus as a triple mutant lacking these three enzymes has been obtained (Carneiro et al. 2004). According to the authors, an expression profile of the genes encoding these three dehydrogenases revealed that the *nde2* and *ndi1* genes are highly downregulated, probably in a coordinated manner, in the late exponential phase of *N. crassa* growth, in contrast to the *nde1* gene. Similar to NDI1, the NDE2 dehydrogenase may contribute to spore germination as *nde2* mutants exhibit a much slower germination rate compared to the wild-type *N. crassa*. The failure to obtain a double mutant (*nde2cI* mutant) lacking both NDE2 and Complex I unexpectedly suggested that NDI1 is unable to compensate for the lack of Complex I in the *N. crassa* mitochondria (Carneiro et al. 2004). Additional studies have surprisingly implied that NDE2 complements Complex I activity. This observation indicates some ways of exchanging the matrix/cytosolic NADH that are present in *N. crassa*. Based on experiments analyzing the Ca^{2+} influence on the mutant strains, it has been proposed that Ca^{2+} somehow allows for the cytosolic NADH to pass the inner mitochondrial membrane (Carneiro et al. 2004; Melo et al. 2001). Whether the observed effects are due to Ca^{2+} regulation of the permeability transition pore (PTP) still remains unknown.

An NDE3 of a predicted molecular mass of ~55 kDa has been localized both in the mitochondria and in the cytosol of *N. crassa* and its sequence shows no typical features of a mitochondrial cleavable targeting sequence (Carneiro et al. 2007). This enzyme has been proven to be anchored in the inner mitochondrial membrane and face the intermembrane space. Similar to NDE2, NDE3 oxidizes both NADH and NADPH. In contrast to the *nde2cI* mutant, the *nde3cI* mutant has been obtained. Despite the fact that NDE2 has been considered to be the primary external NAD(P)H dehydrogenase, the contribution of NDE3 to the cytosolic oxidation of NAD(P)H has been shown to be greater than previously expected. In the *nde3* mutant, the *nde2* expression increases significantly. However, in the wild-type strain, the upregulation of the *nde3* transcript in contrast to the significant downregulation of *nde2* and *ndi1* has been observed from the early to late exponential phase of growth. These data indicate that the activities of particular dehydrogenases could possibly be partially compensated by others during the different stages of *N. crassa* development. These compensations could enable the fungus to adapt to changing environmental con-

ditions, and the dual location of NDE3 may serve as a type of sensing mechanism (Carneiro et al. 2007).

It has been also investigated whether the alternative NAD(P)H dehydrogenases may be a significant source of ROS generation in mitochondria with a branched respiratory chain (Carneiro et al. 2012). Using paraquat, it was found that *N. crassa* strains devoid of one or more alternative dehydrogenases exhibit an increased tolerance to this redox cycling agent compared to the wild-type strain. In particular, the double mutant lacking NDE1 and NDE2 was the most resistant to the reducing agent, although it did not withstand H_2O_2 and heat shock stresses. In addition, decreased levels of ROS have been observed in the *nde1nde2* mutant strain, and among alternative dehydrogenases, NDE2 has been suggested to play a major role in ROS generation in the *N. crassa* mitochondria (Carneiro et al. 2012).

Yarrowia lipolytica

In the obligate aerobic filamentous fungus *Y. lipolytica*, only one gene encoding an alternative dehydrogenase has been identified and designated *YLNDH2* (Kerscher et al. 1999). The YLNDH2 enzyme is located externally in the inner mitochondrial membrane. Deletion of the gene encoding the enzyme has no influence on the *Y. lipolytica* mutant viability. Therefore, other mechanisms responsible for cytosolic NADH oxidation must also be present in the *Y. lipolytica* mitochondria. The phylogenetic analysis of known NADH type II dehydrogenases revealed that a common ancestor of fungal alternative NADH:UQ oxidoreductases originally had an external orientation and that the internal dehydrogenase form, such as that of *S. cerevisiae*, emerged from divergent evolution following an early gene duplication event (Kerscher et al. 1999). Further studies showed that YLNDH2 is engaged in supercomplex formation with Complexes III and IV, depending on the cell growth phase (Guerrero-Castillo et al. 2009, 2012). In mitochondria from high energy-requiring cells in the logarithmic growth phase, most YLNDH2 protein was associated with cytochrome *c* oxidase (Complex IV), and electrons from NADH were channeled to the cytochrome pathway (Guerrero-Castillo et al. 2012). In contrast, in the low energy-requiring, late stationary-growth phase, Complex IV concentration decreased, and the cells overexpressed YLNDH2. Thus, a large fraction of this enzyme was found in a non-associated form. The AOX-sustained pathway was activated at the same time, and ROS production also decreased. These association/dissociation processes of YLNDH2 to Complex IV have been proposed to be a switch that channels electrons either to the energy-conserving cytochrome pathway or the energy-dissipating AOX-sustained pathway. The latter entirely nonproton-pumping electron pathway decreases the reduction level of respiratory chain electron carriers, thus preventing the overproduction of ROS. In *Y. lypolytica* mitochondria, as in *S. cerevisiae* mitochondria, it has been suggested that the formation of supercomplexes promotes the channeling of respiratory substrates and electrons, the sequestration of ROS, and the stabilization of labile, multisubunit complexes (Guerrero-Castillo et al. 2009, 2012).

Aspergillus niger

A. niger is another filamentous fungus, in which alternative dehydrogenases have been described. Filamentous fungi are a very important group of microorganisms that are used in industry (O'Donnell et al. 2011). Biotechnological production processes using these organisms are often highly aerobic, thus implying that the fungal cells are subjected to oxidative stress. The presence and activity of type II dehydrogenases in *Aspergilli* have garnered much interest due to their possible antioxidative role in mitochondria. Basing on homology to other fungi and the availability of *Aspergillus* species genomes, putative *nde* and *ndi* genes encoding alternative external and internal NADH dehydrogenases, respectively, have been found (Li et al. 2011). The analysis of the genomes reveals high levels of conservation and genome synteny across the *Aspergillus* species, indicating that these dehydrogenases play a fundamental role in fungal growth in the natural environment in response to oxidative stress. In addition, the dehydrogenases provide useful tools in industrial bioprocesses.

In *A. niger*, the activity of alternative NADH dehydrogenases increases significantly under oxidative stress conditions, while no changes in ROS concentration have been found (O'Donnell et al. 2011). It has been suggested that under oxidative stress conditions, a decrease in ATP production and a diminished capability for highly energetic processes could be a consequence of the enhanced activity of alternative NADH dehydrogenases. Interestingly, these effects lead to a decrease in cellular viability and subsequently cause earlier senescence and culture death. Additional studies have revealed that inhibition of the alternative NADH dehydrogenases by the most effective inhibitor 7-iodoacridone

4-carboxylic acid (IACA) enhances metabolic activity and almost doubles amount of ATP produced in *A. niger* cells (Voulgaris et al. 2012). Thus, these reports show that the inhibition of the alternative NADH dehydrogenases has noticeable effects on the productivity of a bioprocess under moderate oxygenation conditions. It has been suggested that the application of an alternative NADH dehydrogenase deficient mutant, together with the appropriate fermentation conditions, could be a route to significantly increase the productivity of an industrial fungal bioprocess by increasing the growth rate and consequently reducing the fermentation times while at the same time increasing the energetic efficiency (Voulgaris et al. 2012).

Type II NAD(P)H Dehydrogenases in Protists

Trypanosoma

Parasitic protozoans have been extensively investigated due to the search for new effective drugs for the dangerous diseases they cause. Because mammalian mitochondria do not possess alternative dehydrogenase in the respiratory chain, these proteins have become a specific potential target for disease treatment. The African trypanosome *T. brucei*, the causal agent of sleeping sickness in humans and nagana in cattle, has a dual life cycle in the bloodstream of the mammalian host and the insect vector (Bienen et al. 1991; Hajduk et al. 1992). In the mammalian bloodstream, the trypanosomes exist as dividing long slender forms that lack well-developed mitochondria, cytochromes, and cyanide-sensitive electron transport. In these developmental forms, energy requirements are provided completely by glycolysis using glucose from the blood of the mammalian host (Opperdoes 1987). However, the procyclic forms, present in the midgut of the insect host, possess a single, large mitochondrion containing respiratory chain complexes generally similar to those present in eukaryotes (Hajduk et al. 1992). In 2003, an alternative NADH dehydrogenase from the *T. brucei* procyclic form (NDH2) was isolated and characterized (Fang and Beattie 2003b). The enzyme is rotenone-insensitive and contains noncovalently bound FMN as a cofactor, instead of the FAD usually present in eukaryotic type II dehydrogenases. FMN appears to serve as one electron donor to UQ or oxygen in contrast to the two-electron reduction conducted by FAD. This feature fosters ROS production, since superoxide is generated by a one-electron reduction of molecular oxygen. A previous study confirmed that the rotenone-insensitive NADH dehydrogenase is a potential source of superoxide production in procyclic trypanosome mitochondria (Fang and Beattie 2002b). The *T. brucei* alternative NADH dehydrogenase is a dimer of 65 kDa, which separates into two 33 kDa subunits, and is located on the inner mitochondrial membrane facing the matrix (Fang and Beattie 2003b). Because of a low Complex I activity, one of possible functions for NDH2 in *T. brucei* mitochondria is to complement Complex I, mediating electron transfer from internal NADH to the respiratory chain. Subsequent studies on the requirement for the core subunits of Complex I in the *T. brucei* respiratory chain lead to conclusion that Complex I activity can be fully replaced by NDH2 (Verner et al. 2013). In addition, the enzyme is capable of utilizing deamino NADH and NADPH as substrates in vitro (Fang and Beattie 2003b). Interestingly, the mitochondria of the procyclic *T. brucei* depleted of Complex I subunits exhibit an increased sensitivity of the NADH oxidation to diphenyloiodonium chloride (IDP) and a lower sensitivity to rotenone, a specific inhibitor of Complex I (Verner et al. 2011). However, the depletion of NDH2 affects both cell growth and the mitochondrial membrane potential, although the remaining activities of the respiratory complexes are unaltered with an exception of the increased activity of glycerol-3-phosphate dehydrogenase (Verner et al. 2013). These results support the hypothesis that in the procyclic *T. brucei* mitochondrion, NDH2 might be preferentially used to regenerate NAD^+ and maintain the mitochondrial membrane potential compared to Complex I.

Apicomplexans

Apicomplexans are unicellular and spore-forming obligate intracellular parasites that occupy diverse host niches (Mogi and Kita 2010). They have remodeled mitochondrial carbon and energy metabolism through reductive evolution. The development of novel drugs is now a very serious challenge in the face of the increasing problem of the multidrug resistance of *Plasmodium* that causes malaria in humans. The function of the *Plasmodium* mitochondria is unclear because it is widely accepted that the majority of the energetic demand of the parasite is provided by glycolysis. In addition, it has been suggested for a long time that the *Plasmodium* mitochondria cannot conduct oxidative phosphorylation, because they lack the membrane anchor subunits for ATP synthase. How-

ever, it has been shown that the mitochondria can generate a large transmembrane potential (Biagini et al. 2006). Ten subunits of *Plasmodium* F_oF_1-ATP synthase, including membrane anchor subunits a and b, were finally identified, although they are highly divergent from their eukaryotic and bacterial counterparts (Kawahara et al. 2009). Thus, the *Plasmodium* mitochondria appear to be capable of oxidative phosphorylation. However, it is more likely that the mitochondria of these malaria parasites are engaged in cellular functions other than ATP synthesis, such as calcium homeostasis maintenance or pyrimidine synthesis (Stocks et al. 2014).

There is no evidence for Complex I presence in the *P. falciparum* mitochondrial electron transport chain, but bioinformatic analysis has identified the type II NADH dehydrogenase (PfNDH2) (Gardner et al. 2002). Analysis of the *PfNDH2* gene indicates that the PfNDH2 protein is a single 52 kDa monomer and possesses two regions, one likely responsible for the noncovalent attachment of the flavin nucleotide cofactor and the second probably responsible for NADH-binding (Fisher et al. 2007). The mode of interaction with UQ has not yet been elucidated, but it has been suggested that the enzyme kinetics follows a ping-pong (nonsequential) mechanism. Clustal analysis of PfNDH2 with EF-hand-containing alternative NADH dehydrogenases does not indicate the presence of conserved EF-hand domains. In addition, Ca^{2+} dependence has not been determined experimentally. Kawahara et al. (2009) reported that PfNDH2 reoxidizes NADH in the mitochondrial matrix and can also use NADPH as a substrate. Importantly, the PfNDH2 activity is essential for the generation of mitochondrial transmembrane potential and the inhibition of the dehydrogenase is lethal to *P. falciparum* (Biagini et al. 2006). It is likely that PfNDH2 serves as a "choke point" in the mitochondrial electron transport chain, enabling electron transfer through the other respiratory chain complexes. However, in the *P. falciparum* mitochondria, the proton circuit is different from typical mitochondria, since the primary generators of the electrochemical gradient are Complexes III and IV (Fisher et al. 2007). Although necessary subunits of the ATP synthase are present and assemble correctly in the *P. falciparum* mitochondria, the contribution of the enzyme to ATP generation is imperceptible (Balabaskaran et al. 2011). Because a proton electrochemical gradient exists across the *P. falciparum* inner mitochondrial membrane, there must be a proton leak in the membrane that is sufficient to complete the proton circuit (Fisher et al. 2007). It remains unclear whether it is the basal proton conductance of the membrane, a specific uncoupling protein, or a minimal ATP flux through ATP synthase. It has been hypothesized that PfNDH2 is an evolutionary adaptation to a microaerophilic lifestyle of the parasites enabling the (proton) uncoupled oxidation of NADH and a reduction in mitochondrial superoxide generation (Fisher et al. 2007).

Similar to *P. falciparum*, there is no evidence of the presence of Complex I in *Plasmodium yoelii* mitochondria, but an alternative NADH dehydrogenase with an approximately 65 kDa molecular mass exists in the inner mitochondrial membrane (PyNDH2) (Uyemura et al. 2004). In addition, in these malarial parasites, oxidative phosphorylation is considered minimal if it exists at all. In contrast to *P. falciparum* and other known parasites, the presence of the AOX has been excluded in the *P. yoelii* mitochondria (Uyemura et al. 2004). However, a fatty acid-induced GTP-inhibited mitochondrial uncoupling has been observed, suggesting that the activity of the uncoupling protein (UCP) could account for the closing of the proton circuit in the *P. yoelii* inner mitochondrial membrane (Uyemura et al. 2004).

Cryptosporidium is a protist causing serious diarrhea in humans and other animals (Bouzid et al. 2013). In its mitosomes, the most reduced forms of mitochondria, it is assumed that the membrane potential is generated by a simple respiratory chain consisting of transhydrogenase, type II NADH dehydrogenase, and AOX (Mogi and Kita 2010). In some *Cryptosporidium*, a unique respiratory chain consisting of externally bound NDH2 and internally bound AOX can reoxidize cytoplasmic NADH. ATP and NADPH are produced in the mitosome and could be used for iron-sulfur cluster formation.

The genome of *Toxoplasma gondii*, the parasite that causes toxoplasmosis, encodes two NDH2 isoforms and both are constitutively expressed (TgNDH2-I and TgNDH2-II) (Lin et al. 2008). The two TgNDH2 isoforms are internal enzymes that face the mitochondrial matrix with their active sites. Knockout experiments indicated that both *Tgndh2* genes are required to maintain normal mitochondrial membrane potential and the intracellular ATP level, although TgNDH2-II appears to be more important (Lin et al., 2011). TgNDH2-I has been heterologously expressed in *Y. lipolytica* mitochondria and its kinetic parameters have been determined (Lin et al. 2008). Kinetic studies have revealed that the NADH dehydrogenase activity of TgNDH2-I follows a ping-pong mechanism, a mode of action also shown for the *Y. lipolytica* ortholog and proposed for the *S. cerevisiae* and *T. brucei* enzymes. For TgNDH2-I, the

K_M determined for NADH is equal to 76 μM and is significantly higher than the known K_M values of most other eukaryotic enzymes. For example, they range from 9–56 μM in *N. crassa* (Velazquez and Pardo 2001) and *S. cerevisiae* (Duarte et al. 2003; Melo et al. 2004). The exception is the *T. brucei* enzyme with a K_M of ~120 μM (Fang and Beattie 2002a). However, differences in K_M values have to be interpreted with caution, since different electron acceptors were used during the measurements. TgNDH2-I is inhibited effectively (in nanomolar concentration) by the quinolone-like compound, 1-hydroxy-2-dodecyl-4(1)quinolone (HDQ) and other quinolones possessing longer alkyl side chains. However, it cannot be ruled out whether HDQ inhibits other ubiquinone-dependent oxidoreductases, such as succinate dehydrogenase or glycerol-3-phosphate dehydrogenase, in addition to the alternative NADH dehydrogenases (Fang and Beattie 2002a).

Amoeba

The amoeba *Acanthamoeba castellanii* is a small, nonphotosynthesizing free-living soil and freshwater protozoan that can cause serious diseases in humans, such as *Acanthamoeba* keratitis (AK) and granulomatous amoebic encephalitis (GAE) (Marciano-Cabral and Cabral 2003; Schuster and Visvesvara 2004). The mitochondria of *A. castellanii* possess a branched plant type respiratory chain with AOX and alternative NAD(P)H dehydrogenases (Antos-Krzeminska and Jarmuszkiewicz 2014a, 2014b). Annotated sequences of two putative alternative NAD(P)H dehydrogenases from the protozoan *A. castellanii* demonstrate similarity to plant and fungal sequences and reveal EF-hand motifs indicating Ca^{2+}-binding domains (Antos-Krzeminska and Jarmuszkiewicz 2014a). BN-PAGE electrophoresis and histochemical staining of *A. castellanii* mitochondrial proteins reveal six protein bands of relatively low molecular mass (~50–70 kDa): three with NADH-oxidizing activity, and the other three with NADPH-oxidizing activity. In isolated *A. castellanii* mitochondria, external NADPH oxidation has been observed for the first time in protist mitochondria. In *A. castellanii* mitochondria, external NADH and NADPH oxidation exhibit similar coupling parameters, indicating similar efficiencies of ATP synthesis, and the same optimal pH (6.8) independent of relevant ubiquinol-oxidizing pathways, the cytochrome pathway or a GMP-stimulated AOX-sustained pathway. However, a twice lower maximal oxidizing activity, 10-fold higher Ca^{2+}-dependence, and a lower K_M value for external NADPH oxidation were observed compared to those for external NADH oxidation. The results indicate a higher dehydrogenase affinity for external NADPH, and therefore a finely tuned strong Ca^{2+}-dependent regulation of external NADPH oxidation in the *A. castellanii* mitochondria. Remarkably, external NADH is in addition to succinate (Complex II substrate) one of the strongest respiratory substrates, indicating a substantial contribution of external NADH dehydrogenase activity to the electron transport chain in the *A. castellanii* mitochondria and increased substrate (external NADH) availability in the cytosol (Antos-Krzeminska and Jarmuszkiewicz 2014a).

NADH and NADPH Turnover and the Putative Role of Alternative NAD(P)H Dehydrogenases

Still very little is known about the metabolic function of external and internal alternative NADH:UQ oxidoreductases, and almost nothing is known about the mechanisms underlying their metabolic regulation, especially in fungi and protists. External NADH dehydrogenases are obviously involved in feeding electrons from NADH generated in the cytoplasm into the mitochondrial respiratory chain. Internal alternative NADH enzymes may compete with Complex I for the substrates matrix NADH and UQH_2 (ubiquinol, the reduced form of UQ). Although, electron transport via an internal alternative nonproton-pumping dehydrogenase results in a lower transmembrane potential compared to electron transport via Complex I, the possibility to build (or complement) a functional electron transport chain by the expression of a single polypeptide instead of the at least 35 subunits of Complex I seems to be advantageous under conditions where the carbon source is abundant and rapid growth is essential (Melo et al. 2004). Despite a large amount of new information on NDH2 that has been collected, many questions remain unanswered, especially those concerning the function of the enzymes in cellular metabolism.

Since the pyridine nucleotides are central mediators of the reducing power flow between different cellular processes and compartments (Rasmusson and Wallstrom 2010), the presence of several NDH2 enzymes could possibly improve the catalytic flexibility of respiratory NAD(P)H oxidation and therefore thereby the redox balancing or sensing (Geisler et al. 2007). These factors merge energy metabolism with carbon metabolism and

stress defense. Mitochondrial NAD(P)H oxidation may participate in the prevention of ROS formation (Fernie et al. 2004; Moller 2001) and in decreasing the excess of reducing equivalents feeding the mitochondrial respiratory chain (Raghavendra and Padmasree 2003). However, some data indicate that NDH2 could increase ROS production causing apoptosis of the cell (Carneiro et al. 2012; Fang and Beattie 2002b, 2003a).

The external NAD(P)H dehydrogenases are involved in the modulation of the cytoplasmic NAD(P)H pool. In mitochondria, the major citric acid cycle enzymes and the metabolite exchangers together mediate reducing fuel shuttling across the inner membrane resulting in redox separation between the mitochondrial and cytosol compartments (Moller 2001). The malate/oxaloacetate shuttle maintains a sharp NADH gradient between the cytosol and mitochondrial matrix. Therefore, the primary task in maintaining the NADH redox balance belongs to the internal NADH dehydrogenases including both Complex I and alternative internal dehydrogenases. In turn, the level of NAD(P)H in the cytosol is the effect of a variety of metabolic pathways involved, including the oxidative pentose phosphate pathway (PPP) and the participation of cytosolic NAD(P)H kinases. The NADPH molecule is crucial for many biological pathways such as cellular antioxidative system-mediated reactions (Rasmusson and Wallstrom 2010). Thus, the mitochondrial alternative NAD(P)H dehydrogenases may participate in maintaining the appropriate redox balance. Considering their Ca^{2+} dependence, it has been suggested that some type II dehydrogenases will be inactive in unstressed cells (Moller 2001). Interestingly, in plants, the genes encoding type II NAD(P)H dehydrogenases and the AOX have been demonstrated to be expressed simultaneously under stress conditions and during development (Clifton et al. 2005; Ho et al. 2007; Rasmusson et al. 2009), indicating the coupling of these two alternative pathways.

Alternative NAD(P)H dehydrogenases do not participate in a proton electrochemical gradient generation in the inner mitochondrial membrane. Their action leads to the gradient dissipation. Thus, they are energy-dissipating systems. Because the proton electrochemical gradient powers transport processes through the membrane, it is essential to maintain it at the appropriate level. In addition, mitochondrial ROS production depends on the level of the reduction of mitochondrial electron carriers that is related to the level of the proton electrochemical gradient across the inner membrane (Dominiak et al. 2018). Therefore, by influencing the proton electrochemical gradient, NDH2 may modulate transport processes across the inner membrane and mitochondrial ROS generation. It has been proposed that during evolution, the involvement of the alternative NAD(P)H dehydrogenases in the mitochondrial respiratory chain may be one of mechanisms that enables them to adjust the cellular metabolism to changing environmental conditions and protect against ROS formation (Moller 2001). As described in this review, alternative NAD(P)H dehydrogenases are present in the branched respiratory chain not only in plants but also in some non-photosynthesizing unicellular eukaryotes, including amoeboid protists, as well as in filamentous fungi (Table 1). Interestingly, they are also present in the reduced mitochondrial respiratory chain of fermentative yeast and parasite protists, including apicomplexans.

Reports on the participation of NDH2 along with other respiratory complexes in the formation of a large supercomplex (Grandier-Vazeille et al. 2001) or even a respirosome-like structure in *S. cerevisiae* mitochondria (Matus-Ortega et al. 2015) are very intriguing. Similarly, in *Y. lypolytica* mitochondria, it has been suggested that formation of the supercomplex promotes substrate and electron channeling, ROS sequestering, and the stabilization of labile, multisubunit mitochondrial complexes (Guerrero-Castillo et al. 2009, 2012). In addition, studies on the *N. crassa* NDH2 have indicated a possible partial compensation of particular dehydrogenases by each other in different stages of development, indicating a higher level of regulation of the respiratory chain components (Carneiro et al. 2007). These findings suggest the presence of "respiratory strings", precisely regulated systems in mitochondria, which can both facilitate electrons channeling between respiratory complexes and control the abundance and activities of the respiratory chain components. Therefore, considering the functions of the alternative NAD(P)H dehydrogenases, the general dynamics of the mitochondrial respiratory chain should be taken into account.

It has been suggested that type II dehydrogenases are involved in programmed cell death in fungi. In *A. niger*, a decrease in ATP production as a consequence of enhanced alternative NADH dehydrogenase activity leads to a decrease in cellular viability and subsequently cause earlier senescence and culture death (O'Donnell et al. 2011). In yeast, NDI1 may be involved in the cell death induced by different stimuli, such as hydrogen peroxide, acetic acid, and manganese ions (Cui et al. 2012). Interestingly, alternative NAD(P)H dehydrogenases are phylogenetically related to cell

Table 1. Basic features of type II dehydrogenases in fungi and protozoa.

Organism and dehydrogenases	Location in the inner mitochondrial membrane	Group	Cofactor	Substrate	Calcium dependence	K_M [μM]
FUNGI						
Fermentative yeast						
S. cerevisiae						
NDI1	Internal	A/B	FAD	NADH	nd	32 [Q_6]
NDE1	External	A/B	FAD	NADH	nd	nd
NDE2	External	A/B	FAD	NADH	nd	nd
K. lactis						
KlNDI1	Internal	A	FAD	NADH	–	nd
KlNDE1	External	A	FAD	NADPH	–	66
KlNDE2	External	B	FAD	NADH/NADPH	–	36 (for NADH)
Filamentous fungi						
N. crassa						
NDI1	Internal	A	FAD	NADH	–	56 [Q_2]
NDE1	External	B	FAD	NADPH	+	11
NDE2	External	A	FAD	NADH/NADPH	–	12 (for NADH)
NDE3	External	A	FAD	NADH/NADPH	–	nd
Y. lypolytica						
YlNDH2	External	A/B	FAD	NADH	nd	15
A. niger						
NDI	Internal	nd	FAD	NADH	nd	nd
NDII	External	nd	FAD	NADH	nd	nd
PROTOZOA						
Trypanosoma						
T. brucei						
NDH2	Internal	A	FMN	NADH/NADPH	–	120 (for NADH)
Apicomplexants						
P. falciparum						
PfNDH2	Internal/External?	B	nd	NADH/NADPH	nd	104 [Q_0], 2-16 [Q_1] (for NADH)
P. yoelii						
PfNDH2	Internal	A/B	nd	NADH/NADPH	nd	63 (for NADH)/157 (for NADPH)
Cryptosporidium						
NDH2	External	nd	nd	NADH	nd	nd
T. gondii						
TgNDH2-I	Internal	nd	nd	NADH	nd	61-76 [DBQ]

Table 1 (*Continued*)

Organism and dehydrogenases	Location in the inner mitochondrial membrane	Group	Cofactor	Substrate	Calcium dependence	K_M [μM]
TgNDH2-II	Internal	nd	nd	NADH	nd	nd
Amoeba						
A. castellanii NDE1-2	External	B	nd	NADH/NADPH	+	82 (for NADH)/34 (for NADPH)

Group A contains two nucleotide-biding motifs, the NAD(P)H-biding motif and the flavin nucleotide-binding motif; group B contains the both nucleotide-binding motifs and the EF-hand motif. A/B, A or B, depending on different data (Michalecka et al. 2003; Melo et al. 2004; Fisher et al. 2007). K_M values for NADH/NADPH, if not distinguished, were measured with endogenous electron acceptor of respiratory chain; otherwise, artificial electron acceptors are presented in square brackets. Q_0, Q_1, Q_6, different ubiquinones; DBQ, *n*-decylubiquinon. nd, not determined.

death-promoting proteins of the apoptosis-inducing factor (AIF)-family (Goncalves and Videira 2015).

In apicomplexans, the internal NADH dehydrogenase is suggested to be a critical component of the mitochondrial electron transport chain lacking Complex I (Biagini et al. 2006) and an evolutionary adaptation to a microaerophilic lifestyle of parasites that enable uncoupled NADH oxidation and therefore a lowering of mitochondrial reducing power, as well as a reduction of mitochondrial superoxide generation (Fisher et al. 2007). On the other hand, internal NDH2 has been found to be a potential source of superoxide production in procyclic trypanosome mitochondria (Fang and Beattie 2002b). Recent studies have shown that in *T. brucei* proliferating bloodstream forms, internal NDH2 is advantageous, but not essential, in this parasite stage when the mitochondrial NAD^+/NADH balance is not very important (Surve et al. 2017). Similarly, it has been observed that in *N. crassa*, alternative dehydrogenases are beneficial but not indispensable during fast growth, when carbon sources are abundant in the environment (Carneiro et al. 2012).

Parasitic protists use mitochondria or the mitochondrion-derived organelle (mitosomes) and enzymes for the aerobic (or anaerobic) oxidation of substrates and for energy production (Mogi and Kita 2010). Due to absence of NDH2 in the mammalian mitochondria, alternative dehydrogenases are potential drug targets to treat diseases caused by *Trypanosoma*, parasite amoeba or apicomplexans. Unfortunately, it still remains a large challenge to find specific inhibitors of NDH2 (Dong et al. 2009).

Final Remarks

Alternative NAD(P)H dehydrogenases are present in the branched respiratory chain not only in mitochondria of plants but also in non-photosynthesizing unicellular eukaryotes, including amoeboid protists, as well as in filamentous fungi. NDH2 are also present in the reduced mitochondrial respiratory chain of fermentative yeast and parasite protists, including apicomplexans. The genomic data available indicate that, with the exception of animals, most eukaryotes contain genes that potentially encode NDH2. Their partial conservation throughout evolution and widespread presence makes them a particularly interesting subject of research. The physiological role of the alternative rotenone-insensitive dehydrogenases is still incomprehensible, especially in the mitochondria of fungi and protists. Compared with plant NDH2, the molecular and functional properties, regulation, and physiological impact of fungal and protozoan alternative NAD(P)H dehydrogenases are much less studied and surveyed. It can be assumed that their physiological role lies in the modulation of the mitochondrial energy metabolism in response to stress situations. They may prevent the overreduction of the electron transport chain components and thereby decrease production of ROS. Alternative respiratory pathways, including NDH2, may flexibly change in response to various environmental factors. Therefore, energy-dissipating systems, such as NDH2, may play an important role in the metabolic and energetic

adaptation to the changes in the surrounding environment that occur during the life of fungi and protists.

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