Winter 3-1-2019

Alternative NAD(P)H dehydrogenase and alternative oxidase: proposed physiological roles in animals

Allison McDonald
Wilfrid Laurier University, amcdonald@wlu.ca

Dmytro V. Gospodaryov
Vasyl Stefanyk Precarpathian National University, dmytro.gospodaryov@gmail.com

Alternative NAD(P)H dehydrogenase and alternative oxidase: proposed physiological roles in animals

Allison E. McDonald[1]*
[1]Department of Biology, Wilfrid Laurier University, Science Building, 75 University Avenue West, Waterloo, Ontario N2L 3C5, Canada

Dmytro V. Gospodaryov[2]*
[2]Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenka, Ivano-Frankivsk 76018, Ukraine

*Corresponding authors:
Allison E. McDonald,
phone: +15198840710 x4180; email: amcdonald@wlu.ca,
Dmytro V. Gospodaryov,
phone: +380342596171; email: dmytro.gospodaryov@gmail.com

Abstract

The electron transport systems in mitochondria of many organisms contain alternative respiratory enzymes distinct from those of the canonical respiratory system depicted in textbooks. Two of these enzymes, the alternative NADH dehydrogenase and the alternative oxidase, were of interest to a limited circle of researchers until they were envisioned as gene therapy tools for mitochondrial disease treatment. Recently, these enzymes were discovered in several animals. Here, we analyse the functioning of alternative NADH dehydrogenases and oxidases in different organisms. We propose that both enzymes ensure bioenergetic and metabolic flexibility during environmental transitions or other conditions which may compromise the operation of the canonical respiratory system.

Key words: cyanide-resistant respiration, rotenone-insensitive NADH dehydrogenase, comparative bioenergetics, gene therapy, mitochondrial disease, hypoxia

1. Introduction

The conventional mitochondrial respiratory system (RS) depicted in textbooks is the one found in humans and is composed of several proton-pumping complexes (Fig. 1A). Three of these are multi-subunit transmembrane enzymes of the oxidoreductase class. Complex I oxidizes nicotinamide adenine dinucleotide (NADH) formed in the tricarboxylic acid (TCA) cycle, while reducing ubiquinone to ubiquinol and pumps protons through the inner mitochondrial membrane (IMM) from the matrix to the intermembrane space (IMS). Complex III oxidizes ubiquinol, while reducing cytochrome c, a small hydrophilic protein. At this complex, two protons are taken up from the matrix side and, simultaneously, two protons are released into the IMS. The reduced cytochrome c is then oxidized by proton-pumping cytochrome c oxidase, complex IV. Complex IV transfers electrons from cytochrome c to oxygen and takes up protons from the matrix, producing two molecules of water per one molecule of oxygen. Protons, pumped across the membrane by all of the complexes described, form a proton-motive force across the IMM which tranduces energy for different types of work, particularly for the synthesis of adenosine triphosphate (ATP). Complex V, ATP synthase, allows protons to return from the IMS back into the matrix, using the energy of the proton-motive force for the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate. This ATP is used as an energy source in numerous intracellular processes. Various non-proton-pumping enzymes, able to catalyse ubiquinone or cytochrome c reduction, are also considered to be components of RSs (Fig. 1B). The most familiar one among these enzymes is succinate dehydrogenase (complex II) which oxidizes succinate to fumarate, while reducing ubiquinone. Ubiquinone can also be reduced by mitochondrial *sn*-glycerol-3-phosphate dehydrogenase, class 2 dihydroorotate dehydrogenase, electron-transferring-flavoprotein dehydrogenase, and several other enzymes (Lenaz and Genova, 2010; Fig. 1B).

The RS can be branched or modified at points of ubiquinone reduction and ubiquinol oxidation. The branching at the point of ubiquinone reduction is mediated by alternative (or type II) NADH dehydrogenases (NDH2s), single- or oligo-subunit enzymes which catalyse the same reaction as complex I but do not pump protons across the IMM (Melo et al., 2004; Rasmusson et al., 2004; Iwata et al., 2012; Matus-Ortega et al., 2015). These enzymes are not inhibited by rotenone, a classic inhibitor of complex I. They contain flavin adenine dinucleotide cofactor (FAD), unlike the flavin mononucleotide (FMN) and iron-sulphur (Fe-S) centres found in complex I. Finally, NDH2s are not transmembrane proteins and do not

cross through the IMM; they are associated with either the matrix side (internal NDH2) or the IMS side (external NDH2) of the IMM. The relatives of NDH2s are thioredoxin reductase, dihydrolipoamide dehydrogenase, and some other flavin-containing dehydrogenases (Kerscher, 2000; Dym and Eisenberg, 2001; Kerscher et al., 2008; Ojha et al., 2007). Notably, a relative of NDH2, sulphide:quinone oxidoreductase (Fig. 1B) is a non-classical RS enzyme which uses reductants (sulphide, sulphite, or cyanide) which are not normally formed under aerobic metabolism in typical eukaryotes (Hildebrandt and Grieshaber, 2008). Some distant homology is observed between NDH2s and apoptosis-inducing factor (AIF), and apoptosis-inducing factor-like proteins (Elguindy and Nakamaru-Ogiso, 2015; Marreiros et al., 2016). Moreover, it was shown that NDH2 from the budding yeast *Saccharomyces cerevisiae* is able to promote apoptosis (Li et al., 2006; Cui et al., 2012), while AIF can operate as a NADH dehydrogenase (Elguindy and Nakamaru-Ogiso, 2015).

Respiratory system branching may also occur at the point of ubiquinol oxidation, by using the enzyme alternative oxidase (AOX). AOX catalyzes oxidation of ubiquinol and reduction of oxygen to water. In the reaction catalysed by AOX, unlike the cytochrome c oxidase reaction, oxygen acquires protons taken from ubiquinol but not from the mitochondrial matrix (Young et al., 2016). AOX is not inhibited by cyanides which are frequently used as inhibitors of cytochrome *c* oxidase. Instead, it is inhibited by salicylhydroxamic acid and alkylated (*n*-propyl- and octyl- are most used) gallates (Rogov et al., 2014). The enzyme contains two iron atoms incorporated to the polypeptide via coordination with carboxylic groups of glutamic acid residues (Berthold and Stenmark, 2003; Young et al., 2016). The relatives of the enzyme are plastoquinol terminal oxidase, dimetoxyquinone hydrolase, ribonucleotide reductase, and a few other proteins (Berthold and Stenmark, 2003).

Several other proteins can be components of alternative RSs, for instance, flavocytochrome b_2 (L-lactate:cytochrome c oxidoreductase) in the budding yeast (Brooks, 2002) or the bacterial cytochrome *bd* terminal oxidase (Borisov et al., 2011). However, the latter two enzymes are not present in animal mitochondria. Therefore, we omit discussion of these proteins in this review, while focusing only on NDH2s and AOXs.

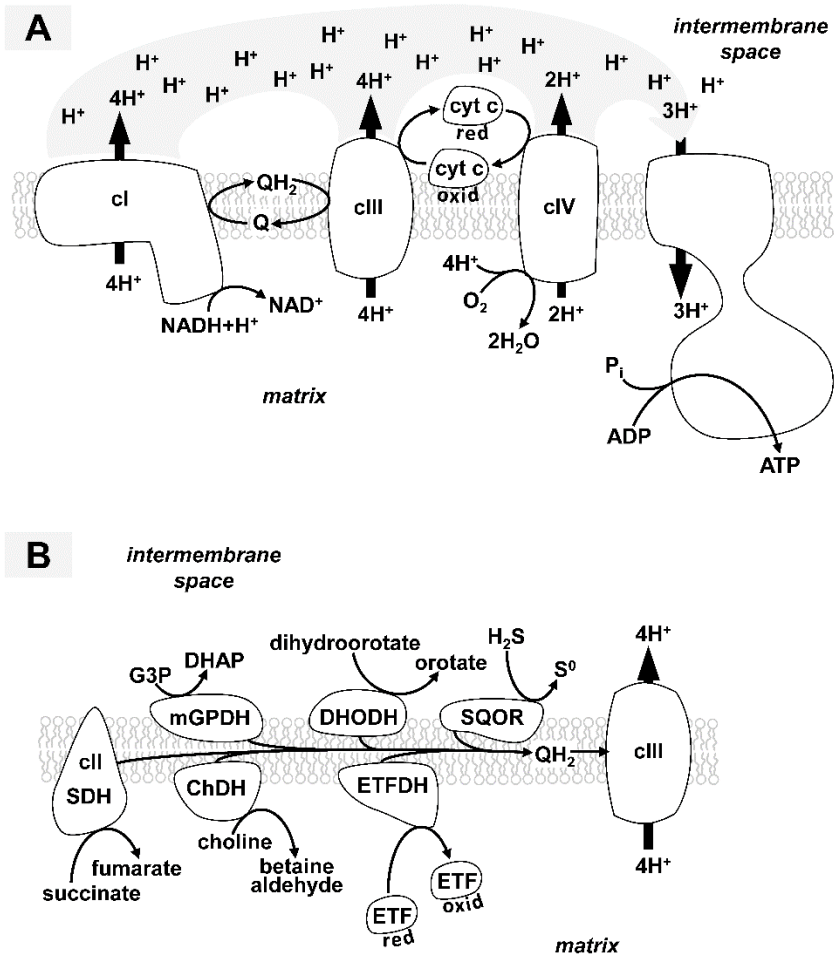

Fig. 1. (A) The canonical respiratory system composed of three proton-pumping multi-subunit complexes (complex I, complex III, and complex IV), which pump protons from the mitochondrial matrix to intermembrane space, while complex V (mitochondrial ATP synthase) passes protons back to the matrix, using their energy for ATP synthesis. (B) Complex II (succinate dehydrogenase; SDH) of the respiratory chain along with a number of other enzymes contributes to the reduction of the ubiquinone pool. Additional enzymes, which catalyse ubiquinone reduction are mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH), class 2 dihydroorotate dehydrogenase (DHODH), sulphide:quinone oxidoreductase (SQOR), mitochondrial choline dehydrogenase (ChDH), and the electron-transferring flavoprotein dehydrogenase (ETFDH).

Until recently, the branching of the conventional RS was deemed to be limited to such organisms as archaea, bacteria, plants, fungi, and protozoans. At the time it was believed that NDH2 and AOX were absent in animals. Extensive sequencing of animal genomes in the past two decades and their subsequent analysis discovered genes coding for non-conventional RS

enzymes in some phyla. Since then, sequences encoding NDH2s and AOXs have been found in the genomes of various animal phyla, including *Placozoa*, *Porifera*, *Cnidaria*, *Annelida*, *Echinodermata*, *Mollusca*, *Nematoda*, *Hemichordata*, and *Chordata* (McDonald and Vanlerberghe, 2004; McDonald et al., 2009; Matus-Ortega et al. 2011). The discovery now serves as a cautionary tale against blindly accepting dogma, but at the time it was controversial. On one hand, the phenomenon could merely represent DNA originating from contamination of these sequences by endosymbionts due to poor sample preparation. Alternatively, the presence of these sequences might be due to a recent lateral gene transfer from algal, fungal, or microbial symbionts to the genome of the animal. In this case, the gene product may or may not function. If the enzyme is non-functional, the gene encoding it would accumulate mutations. Finally, these enzymes might be encoded by the genomes of the animals themselves and the result of vertical inheritance; these alternative RS enzymes may function in living animals or might only be activated under very specific conditions. Research in a variety of experimental systems now points to the genes being encoded in the animal genomes and indicates that the proteins are catalytically active (Dassa et al., 2009a; Dassa et al., 2009b, Gospodaryov et al., 2014; Robertson et al., 2016). This review synthesizes known aspects related to the function of AOXs and NDH2s in different organisms with an emphasis on their possible roles in animals.

2. Alternative respiratory systems: lessons from bacteria, fungi, and plants

2.1. Alternative respiratory enzymes in bacteria

Notably, NDH2s are observed in many bacterial taxa (Melo et al., 2004; Kerscher et al., 2008; Matus-Ortega et al., 2011; Heikal et al., 2014; Marreiros et al., 2016). The enzyme is often found in facultative anaerobes like *Escherichia coli*, as well as in both obligate aerobic (e.g. mycobacteria) and obligate anaerobic bacteria (e.g. *Bacteroides*). However, NDH2 is absent in certain microaerophilic bacteria or obligate parasites like *Chlamydia pneumoniae*, *Helicobacter pylori*, *Campylobacter jejuni*, *Neisseria meningitidis*, and others (Melo et al., 2004). The presence of NDH2 in some groups of bacteria and its absence in others could be the result of divergence of these groups long ago in evolutionary history. Interestingly, the facultatively anaerobic ethanol-producing bacteria *Zymomonas mobilis* do not contain complex I, but use NDH2 to respire (Kalnenieks et al., 2008). The same strategy is seen in specific yeasts that have lost complex I. This represents an example of parallel evolution of the RS in both prokaryotic and eukaryotic organisms performing ethanol fermentation.

Similarly to NDH2, AOX is also present in bacteria. Bacterial species, which contain AOX may or may not also contain NDH2. It looks like it is not necessary for an organism to have both enzymes in one RS. It implies that NDH2 and AOX may operate separately in different metabolic contexts. For instance, bacteria of the genus *Chlamydia* contain AOX but lack NDH2 (Melo et al., 2004). Facultative anaerobes like those from the genus *Vibrio* contain both NDH2 and AOX (McDonald and Vanlerberghe, 2006; Albury et al., 2010). Moreover, it was shown that AOX is able to support respiration during stress caused by nitric oxide, when the heme-containing ubiquinol oxidase CydAB is inhibited (Dunn et al., 2010).

2.2. Alternative respiratory enzymes in fungi

While it is common for branched RSs to occur in bacteria and archaea (Poole and Cook, 2000), systems containing either NDH2s and/or AOXs have only been more recently discovered in eukaryotes. Internal and external NDH2s were first identified in plants and unicellular fungi. Moreover, some species of facultatively anaerobic yeasts, *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, *Kluyveromyces marxianus*, *Ashbya gossypii*, *Candida glabrata* and other species from the *Saccharomyces*/*Kluyveromyces* cluster do not contain complex I (Kerscher, 2000; Gabaldón et al., 2005; Marcet-Houben et al., 2009), having instead only different types of NDH2s. It is proposed that these fungi lost the complex during evolution (Marcet-Houben et al., 2009). The relevance for such a loss is still poorly understood, although the loss of complex I genes is considered to be an adaptation to a fermentative anaerobic lifestyle (Kerscher, 2000; Marcet-Houben et al., 2009). Instead of complex I, budding yeast possess three NDH2s: Ndi1p (internal), and Nde1p and Nde2p (external). Ndi1p was shown to be necessary in aerobic conditions, especially when ethanol is used as a respiratory source (Kerscher, 2000). External NDH2s were shown to use cytosolic NADH derived from glycolysis (Kerscher, 2000).

Separate operating of AOX and NDH2 is also found in fungi. *Saccharomyces cereivisae* contains several types of NDH2 while no AOX. On the other hand, *Podospora anserina*, *Yarrowia lypolytica*, and many other fungi contain both enzymes (Rogov et al., 2014).

2.3. Factors which may favour the retention of alternative respiratory systems in bacteria and fungi

It is worth reflecting that life on Earth arose under anoxic conditions (Tostevin et al., 2016). Initially, the presence of oxygen would have been toxic or detrimental and ways of dealing with it would have been necessary for survival for species that were obligate anaerobes. In contrast, facultative anaerobes and obligate aerobes would have had to adapt to changes in oxygen concentrations (i.e. periods of hypoxia or anoxia, followed by a return to normoxia). In this way, changes in oxygen levels might cause selective pressure for microorganisms to develop alternative respiratory proteins. Oxygen is a substrate in the formation of toxic reactive species such as singlet oxygen, superoxide anion-radical, hydrogen peroxide, and hydroxyl radical. Complexes I and III are involved in the production of reactive oxygen species (ROS) (Murphy, 2009; Bleier et al., 2015) and ironically may also be targets of ROS (Bleier et al., 2015; Caito and Aschner, 2015). Both complex I and complex III contain iron-sulphur clusters which react with the superoxide anion-radical. Complex I is also susceptible to oxidation of its thiol groups. Changes in oxygen level may augment ROS production by complexes I and III via reverse electron transport as the electron transport system becomes over-reduced in anoxic conditions (Storey, 2004; Loor et al., 2011). Oxygen resupply, which may happen right after anoxia, leads to the uncontrolled passing of electrons from the ubisemiquinone anion-radical, a ubiquinone intermediate formed during quinone reduction/oxidation, to oxygen (Fig. 2). Thus, AOX and NDH2s may serve as emergency enzymes, preventing ROS production during the transition from oxygen-limited conditions to normal oxygen supply. It is suggested that complex I of the canonical RS may have two sites of superoxide production, the flavin group of flavin mononucleotide (FMN) and the ubiquinone-binding site (Jastroch et al., 2010; Fig. 2A and Fig. 2B). The production of superoxide at the flavin site under reoxygenation is promoted by NADH accumulation during the preceding bout of hypoxia (Dröse et al., 2016). At the same time, the build-up of ubiquinol during hypoxia can fuel superoxide generation at the quinone-binding site during reoxygenation by means of reverse electron transport (Murphy, 2009). NDH2 and AOX may help in both situations: NDH2 may decrease the NADH concentration, while AOX may oxidize ubiquinol during oxygen resupply (Fig. 2).

Conceivably, NDH2s could also temporarily substitute for complex I and AOX for complexes III and IV if they are damaged. The synthesis and assembly of complex I is energetically and structurally costly. For instance, synthesis of additional Fe-S clusters would require iron, reduced protein thiols such as glutaredoxin, reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Stehling and Lill, 2013), and other co-factor molecules.

These costs seem to be unfavourable in the case of a predominantly fermentative lifestyle and could be the driving force behind the loss of complex I genes over time.

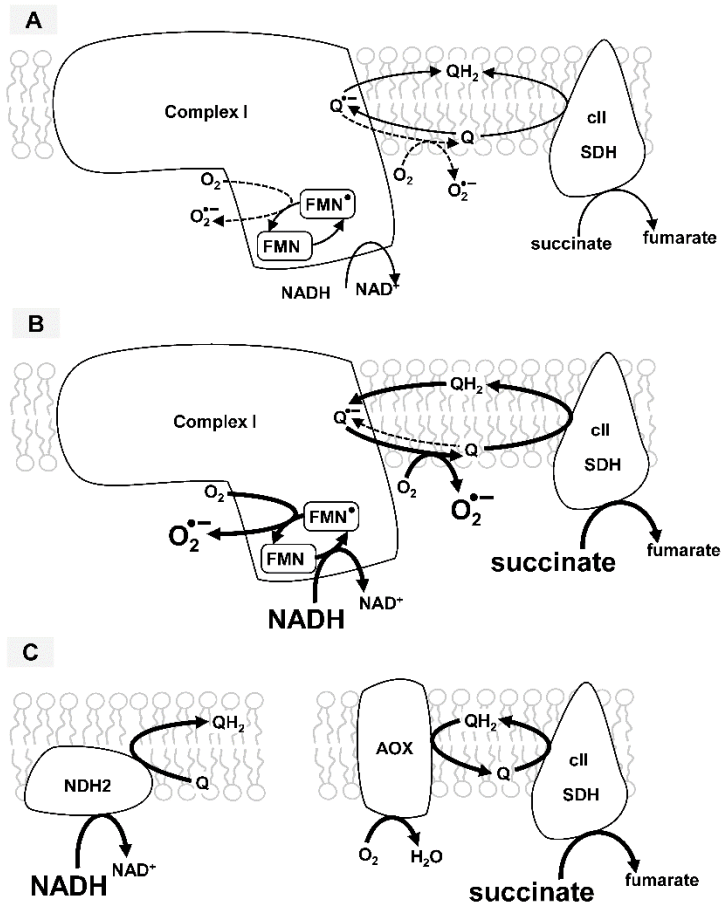

Fig. 2. The role of alternative NADH dehydrogenase and alternative oxidase in maintenance of functional respiratory chain during reoxygenation after anoxia. At normoxia complex I produces low levels of superoxide anion-radical (A). During anoxia complex I is deactivated (Dröse et al., 2016), therefore the NADH/NAD^+ ratio increase. At the same time, succinate dehydrogenase may operate in reverse mode leading to an accumulation of succinate (Chouchani et al., 2016). During reoxygenation, complex I may generate high levels of superoxide at the flavin site via extensive oxidation of accumulated NADH, as well as at the ubiquinone-binding site via reverse electron transport (B). NDH2 may help to oxidize the surplus of NADH while AOX may help to oxidize ubiquinol during reoxigenation (C).

When facultative anaerobes return to aerobic conditions and metabolism, they would require a temporary “crutch” enzyme that is quick and cheap to produce. This enzyme could substitute for a multi-subunit complex during the transition from anaerobic metabolism to an aerobic one. For complex I, NDH2 is an ideal “crutch” because it has a single subunit and requires a relatively simple cofactor, FAD, which is abundant in the anaerobic cell. The same logic can be applied for AOX. This single subunit enzyme can be a monomer or homodimer. It does not require complex cofactors or prosthetic groups, containing only atoms of iron coordinated by residues of glutamic acid (Berthold and Stenmark, 2003).

2.4. Adaptive value of alternative respiratory systems in plants

Plant mitochondria contain multiple types of NDH2s (with either NADPH or NADH specificity, as well as internal or external IMM localization) and AOX. Similar to fungi, many land plants are occasionally subjected to hypoxic conditions (e.g. during overwatering or waterlogging) (Szal et al., 2003; Juntawong et al., 2014). More prolonged anaerobiosis is characteristic for aquatic plants and algae. In these cases, alternative enzymes like NDH2 and AOX may play a role as temporary substitutes for multi-subunit complexes I and III during the transition from hypoxic to normoxic conditions.

Plants have more robust antioxidant systems compared to those of animals as they synthesize a broad spectrum of low molecular antioxidants (e.g. ascorbic acid, carotenoids, tocopherols, phylloquinone, flavonoids, etc.), and several types of peroxidases which use different reductants as substrates. The requirement for such a robust antioxidant system can be accounted for by the intensive generation of ROS in plant cells by the operation of both the mitochondrial and plastid electron transport systems.

Plants are sedentary organisms, unable to physically avoid stresses by changing location. Multiple stresses like hypersalinity, drought, or excess light, affect all cellular compartments, including mitochondria. It was shown that any of the stress conditions listed above triggers oxidative stress (Lushchak, 2011). Thus, complexes of the mitochondrial RS can be damaged by ROS formed during respiration, photosynthesis, or other metabolic processes like the oxidation of D-amino acids. The synthesis and assembly of multi-subunit complexes, their cofactors, and prosthetic groups would require many cellular resources. It appears to be adaptively useful to temporarily substitute complex I or complex III with single-subunit non-proton-pumping alternative enzymes during oxidative stress. The price for this substitution could be a decrease in the proton-motive force and a consequent lowering of ATP production. However, the consequences of a transient drop of ATP production drop via a restriction in the number of proton-pumping units may not be as dramatic as the ATP expenses for the synthesis of multi-subunit protein complexes which are unable to function properly under the given conditions. Moreover, an increase in mitochondrial ROS levels may lead to opening of the permeability transition pore and subsequent cell death via necrosis or apoptosis (Kim et al., 2003).

Alternative respiratory enzymes can also be used by plants and fungi for multiple purposes not directly related to electron transfer. For instance, some plants use AOXs for thermogenesis (Watling et al., 2006; Onda et al., 2008; Ito et al., 2011), and/or adaptation to stresses (Saha et al., 2016; Vanlerberghe et al., 2016; Dahal & Vanlerberghe, 2017). The role of alternative RS enzymes in the regulation of ATP synthesis has also been proposed (Fernie et al., 2004). The tricarboxylic acid (TCA) cycle is not only a generator of reducing equivalents such as NADH, but also provides important intermediates for amino acid synthesis (oxaloacetate, α-ketoglutarate), heme synthesis (succinyl-CoA), fatty acid synthesis (citrate), etc. (Sweetlove et al., 2010). On the other hand, cellular requirements for these intermediates would increase NADH production and subsequently ATP synthesis. In turn, ATP, if not used by various processes in cells, may inhibit glycolysis via phosphofructokinase and pyruvate kinase. ATP will also inhibit AMP-activated protein kinase, affecting energy state metabolism (Storey, 2004).

Organisms which have an alternative RS may by-pass the obstacle of "compulsory" ATP synthesis by lowering the number of proton-pumping points in the RS (Fig. 3). Indeed, the by-passing of complex I by internal NDH2 leaves only two proton-pumping points (Fig. 3B), while the by-pass of complexes III and IV leaves only I proton-pumping complex (Fig. 3C) (Fernie et al., 2004). If both NDH2 and AOX are used, no protons would be pumped across the IMM (Fig. 3D). This mechanism would allow for the fine tuning of ATP production and the degree to which it is coupled to oxygen consumption.

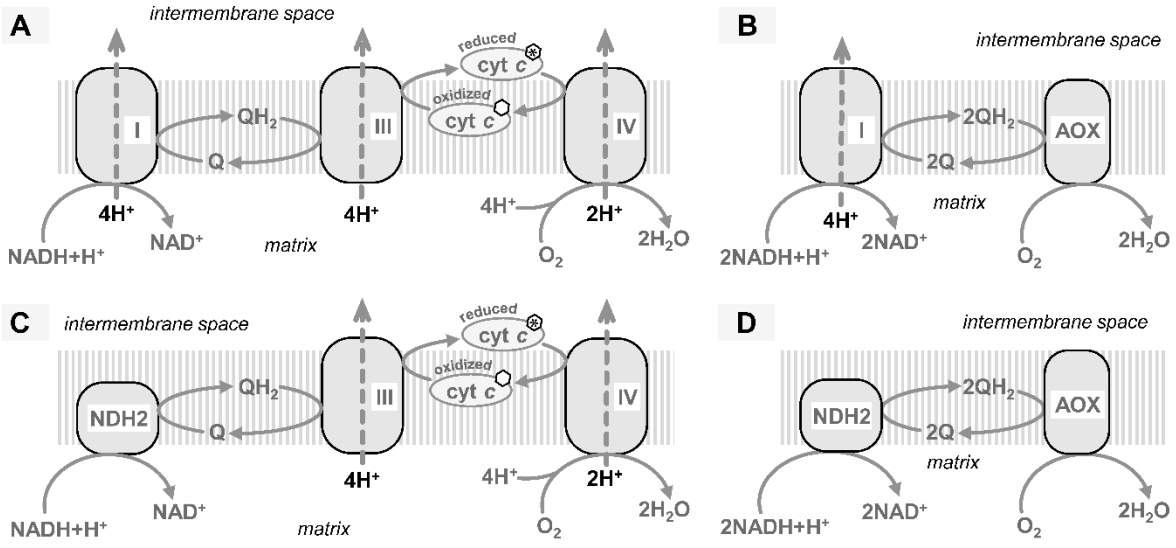

Fig. 3. Enzymes of the branched respiratory system may prevent "compulsory" ATP synthesis in the case of increased metabolic flow through tricarboxylic acid cycle and NADH overproduction. (A) "Normal" operating of the respiratory system, (B) Operating with one proton-pumping unit (complex I) when AOX provides a by-pass of complexes III and IV, (C) Operating with two proton-pumping units when complex I of the canonical respiratory chain is replaced by an alternative NADH dehydrogenase, (D) A respiratory system which does not pump protons across the IMM.

3. The alternative respiratory system in several animal taxa: an explanation of the selective advantage

3.1. Occurrence of NDH2 in animal phyla

The presence of NDH2 in genomes of marine invertebrate animals was recently noted (Matus-Ortega et al., 2011). It is important to differentiate between NDH2 and its paralogs, apoptosis-inducing and apoptosis-inducing-like factors which are also present in humans and may exhibit NADH dehydrogenase activity (Marreiros et al., 2016). However, apoptosis-inducing factors are most likely not constituent components of mitochondrial bioenergetic machinery. So far, the only animal NADH dehydrogenase investigated experimentally is Ndx from *Ciona intestinalis* (Gospodaryov et al., 2014). The Ndx is likely an internal NADH dehydrogenase since it provides rotenone-insensitive respiration in fruit flies, but does not respond to external NADH (D.V. Gospodaryov, unpublished observations). The enzymatic activity of the Ndx with NADH and decylubiquinone as substrates was not detected while functionality of the enzyme was confirmed by polarography (Gospodaryov et al., 2014). It suggests that NDH2 from *C. intestinalis* may be specifically activated in intact mitochondria or use a particular quinone type as substrate. The amino acid sequence of *C. intestinalis* NDH2 has 56% identity with a similar enzyme encoded by *Trichoplax adhaerens,* the simplest multicellular animal. Genes which encode similar enzymes can be found in phyla *Cnidaria* (*Acropora digitifera*, *Aiptasia pallida*, *Hydra vulgaris*, *Nematostella vectensis*), *Porifera* (*Amphimedon queenslandica*), *Priapulida* (*Priapulus caudatus*), *Brachiopoda* (*Lingula anatina*), *Annelida* (*Capitella teleta*), *Mollusca* (*Aplysia californica*, *Biomphalaria glabrata*, *Crassostrea gigas*, *Lottia gigantea*), *Echinodermata* (*Strongylocentrotus purpuratus*), and *Hemichordata* (*Saccoglossus kowalevskii*). Interestingly, in accordance with recent sequencing data, genes coding for putative NDH2s are found also in some tardigrade and spider species, and the Atlantic horseshoe crab (*Limulus polyphemus*), although until recently it was unknown whether *Ecdysozoa,* including tardigrades and arthropods, have

NDH2. The identity of all these sequences with the sequence of the *C. intestinalis* Ndx is about 55-60% (Matus-Ortega et al., 2011; Gospodaryov et al., 2014).

C. intestinalis is phylogenetically the closest organism to humans among those known to possess NDH2. It was tempting to believe that Ndx would rescue complex I defects better than its counterpart from the budding yeast. Unlike Ndi1 from the budding yeast *S. cerevisiae*, Ndx of *C. intestinalis* was not able to rescue defects in subunits of mitochondrial complex I (Gospodaryov et al., 2014). Nevertheless, Ndx caused other physiological effects in the fruit fly model, implying that enzyme requires an intact canonical RS and can be activated under specific conditions (Gospodaryov et al., 2014; Gospodaryov et al., 2016).

3.2. Occurrence of AOX in animal phyla

The data available on cyanide-resistant respiration in molluscs suggested the presence of an alternative RS in their mitochondria (Abele et al., 2007). The presence of AOX genes in animals was discovered over a decade ago and expression at the mRNA level was originally demonstrated in several tissues in *Crassostrea gigas* (Pacific oyster) (McDonald and Vanlerberghe, 2004). Numerous DNA, RNA, and genome sequences obtained in recent years have suggested that a branched RS may function in the mitochondria of several species in different animal phyla (McDonald and Vanlerberghe, 2004; McDonald et al., 2009; Matus-Ortega et al., 2011). Later work using bioinformatics showed that AOX genes were present in nine different animal phyla: *Placozoa, Porifera, Cnidaria, Mollusca, Annelida, Nematoda, Echinodermata*, *Hemichordata*, and *Chordata* (McDonald et al., 2009). It has been known for quite some time that AOX is present in the simplest multicellular animal *Trichoplax adhaerens* (phylum *Placozoa*) and that it is also present in several members of the phylum *Chordata* (McDonald et al., 2009). Since that time, the amount of sequence data deposited in databases has increased exponentially given the use of new sequencing technologies.

A recent search of various databases at the National Center for Biotechnology Information (NCBI) demonstrates that AOX is broadly distributed across many animal phyla (Table 1). The vast majority of new sequences have been identified in members of *Eumetazoa*. Although there has been recent debate about which phylum is more basal, AOX was found in both Cnidarians and Ctenophores (Ryan et al., 2013; Table 1). Within the *Bilateria*, AOX sequences were detected in the phylum Platyhelminthes (Table 1). In addition, many Protostomes and Deuterostomes contain AOX. Within the *Ecdysozoa*, the discovery of AOX

sequences in *Scalidophora* and *Panarthropoda* is novel (Table 1). In contrast to previous reports of the absence of AOX in the phylum *Arthropoda*, AOX genes were found in members of the *Chelicerata*, *Hexapoda*, and *Crustacea* (Table 1). AOX sequences were also found in tardigrades. Within the *Lophotrochozoa*, novel AOXs were found in members of *Brachiopoda* and *Rotifera* (Table 1). Within the Deuterostomes, the presence of AOX was detected for the first time in members of the *Asterozoa* and *Crinoidea* within the phylum *Echinodermata* (Table 1).

The discovery of these novel animal AOXs increases support for the hypothesis that the loss of AOX from some animal lineages (e.g. *Vertebrata*) is most parsimoniously explained as a gene loss event. The taxonomic distribution of AOX in animals known to date indicates that the presence of AOX is an ancestral character (McDonald et al., 2009). While these new data are exciting, they must be interpreted with a degree of caution, and further experimental work must be conducted to ensure that these sequences represent *bona fide* animal sequences. Many of the animals that contain AOX sequences house parasites, symbionts, and/or commensal organisms within or upon their tissues. Many of these new sequences are the result of whole genome sequencing or transcriptome shotgun assembly projects that likely contain contaminating DNA from other organisms. As an example, one sequence recovered from a cnidarian and one from an insect were clearly AOXs of higher plant origin (A.E. McDonald, unpublished data). In addition, the sequences recovered are highly biased towards what species are currently represented in public databases. Gaps exist in our knowledge due to no data being available for many animal phyla and species, especially those that are less accessible and less tractable in the laboratory.

3.3. Co-occurrence and co-expression of NDH2 and AOX in animals

The data on expression of AOX and, especially, NDH2 mRNA and proteins in animals are extremely scarce. At present, we can only guess whether the genes coding for these enzymes are expressed constitutively or as needed. In the case of constitutive expression, it is unknown whether these enzymes are subject to post-translational regulation (e.g. activated/deactivated by a covalent modification (e.g. phosphorylation)) or allosteric regulators. Present data demonstrate that many marine invertebrates exhibit cyanide-resistant respiration (McDonald and Vanlerberghe, 2004; Abele et al., 2007; Ekau et al., 2010; Pichaud et al., 2012; Sussarellu et al., 2013). High levels of AOX mRNA expression were found in heart, hemolymph, and mantle of Pacific oyster (McDonald and Vanlerberghe, 2004). In Eastern oyster, *Crassostrea*

virginica, there are two splice forms of AOX mRNA which are expressed in a number of tissues (Liu and Guo, 2017). Some animals which have an AOX gene also contain the NDH2 gene. This includes animals from phyla *Placozoa*, *Mollusca*, *Echinodermata*, *Cnidaria*, *Porifera*, *Annelida*, and *Chordata.* Some data on expression of NDH2 and AOX proteins in the tunicate *C. intestinalis* can be found in the Ciona intestinalis protein database (CIPRO) (Endo et al., 2011). In accordance with the information present in the CIPRO database, AOX and NDH2 can be simultaneously expressed in hemocytes and embryos of *C. intestinalis*. In contrast, heterologous expression of *C. intestinalis* NDH2 and AOX in fruit flies slightly compromised development (Gospodaryov et al., 2014).

3.4. Factors promoting retention of genes coding for NDH2s and AOXs in animals

3.4.1. Stress conditions and changes in oxygen level

The functional role of the alternative RS in animals is obscure. However, organisms which possess alternative RS components share similarities in their life histories. One common feature shared by these animals is a sessile lifestyle (Matus-Ortega et al., 2011). The animals whose mitochondria contain alternative RS enzymes are sessile, slow moving, or have immobile stages in their life cycle (e.g. many cnidarians, sponges and tunicates). Another common feature of these organisms is their resistance or tolerance to certain stresses, including hypoxia or anoxia, hypersalinity, dehydration, extreme heat or cold, lack of nutrients, exposure to toxicants, etc. For instance, many bivalve molluscs, which were found to possess a branched RS, including *Arctica islandica*, *Crassostrea gigas*, and *Mytilus edulis*, exhibit oxyconformity, an ability to rapidly acclimate to changes in partial oxygen pressure (Abele et al., 2007; Ekau et al., 2010; Rivera-Ingraham et al., 2013; Sussarellu et al., 2013). Short or more prolonged exposure to hypoxic conditions seems to be common for all animals possessing either NDH2 or AOX, or both enzymes. One of these animals, *Trichoplax adhaerens*, is a single representative of *Placozoa* (the simplest multicellular animal) and was found to contain signalling mechanisms responsive to hypoxia (Loenarz et al., 2011). Cnidarians, sponges, echinoderms, bivalve molluscs, and tunicates are intertidal or benthic organisms which are regularly exposed to hypoxia followed by reoxygenation (returning to a physiologically normal oxygen level) (Vaquer-Sunger and Duarte, 2008; Torre et al., 2014). Of note, hypoxia can also be a side consequence of other environmental changes such as increased temperature or salt concentration of water. Both warming and hypersalinity decrease concentration of oxygen dissolved in water, promoting hypoxia (Deutsch et al., 2015).

Recent data indicate the presence of the alternative RS enzymes in several gastropod molluscs (McDonald et al., 2009) which are not as sluggish or immobile as filter feeders. Thus, restricted mobility of an organism is likely not the only trait responsible for the selective pressure to retain genes coding for NDH2s and AOXs. Here, we hypothesize that frequent transitions from hypoxia to reoxygenation which occur during the life history of the organism may be a trait favouring RS branching. As we mentioned above, the hypoxic/normoxic transition is associated with an increase in ROS production by the RS. This situation is very reminiscent of what happens during ischemia, myocardial infarction, and stroke in humans (Chouchani et al., 2016). Perhaps NDH2s and AOXs help marine animals to survive transitions from anaerobic to aerobic conditions without substantial damage caused to membrane lipids and proteins by ROS.

3.4.2. Assembly of co-factors and chemical modifications of canonical RS complexes

Hypoxia/reoxygenation itself would not be the only selective force for retaining alternative RS components. Any condition which may compromise the proper assembly and functioning of multi-subunit RS complexes could be such driver. Regular, but not occasional, lack of iron, sulphur, riboflavin, and proteins and metabolites for Fe-S cluster assembly (glutaredoxins, thioredoxins, NADPH) may be compromising for RS function (Fig. 4). Regular instability of iron-sulphur clusters (e.g. regular oxidative bursts) may also favour the retention of genes coding for NDH2s and AOXs which would bring a selective advantage to the organism in such environments. Parasitic protists and several nematode species are good examples of such a strategy, since they are subjected to regular attacks from the immune system of plant or animal hosts. This attacking of alien organisms by ROS or reactive nitrogen species (nitric oxide) is very common for cells responsible for immune defence in animals (macrophages and neutrophils) and plants. Iron-sulphur clusters of the conventional RS are particularly sensitive to ROS damage. Moreover, assembly of these clusters requires NADPH and glutaredoxins, which provide defence against ROS (Stehling and Lill, 2013). Therefore, the assembly of Fe-S clusters may be compromised during ROS attacks, and may represent a bottleneck for the proper assembly of complexes I and III. Substitution of these complexes by alternative RS enzymes may serve to maintain the functioning of the RS and its associated metabolic processes.

Enzymes of the alternative RS can also be switched on in conditions when the activities of other RS complexes are suppressed or inhibited. For example, complex I and complex IV of

the conventional RS were shown to be nitrosylated, S-nitrosated (Brown and Borutaite, 2004; Burwell et al., 2006; Dahm et al., 2006; Collman et al., 2008; Shiva, 2010; Handy and Loscalzo, 2012; Sarti et al., 2012), reversibly glutathionylated (Taylor et al., 2003; Handy and Loscalzo, 2012; Caito and Aschner, 2015; Picklo et al., 2015; Mailloux and Treberg, 2016), and irreversibly glycated (Rabbani and Thornalley, 2008; Pun and Murphy, 2012; Pun et al., 2014) (Fig. 4). Complex I was shown to be inhibited by glutathionylation (Taylor et al., 2003; Handy and Loscalzo, 2012), while complex IV is inhibited by binding nitric oxide ($^{\bullet}NO$) to haem a_3 (Collman et al., 2008; Sarti et al., 2012) (Fig. 4). Interestingly, protein nitration takes place during the development of sea squirt *Ciona intestinalis* embryos (Ercolesi et al., 2012). Notably, $^{\bullet}NO$ is released during hypoxia and serves also a second messenger during hypoxic signalling (Shiva, 2010).

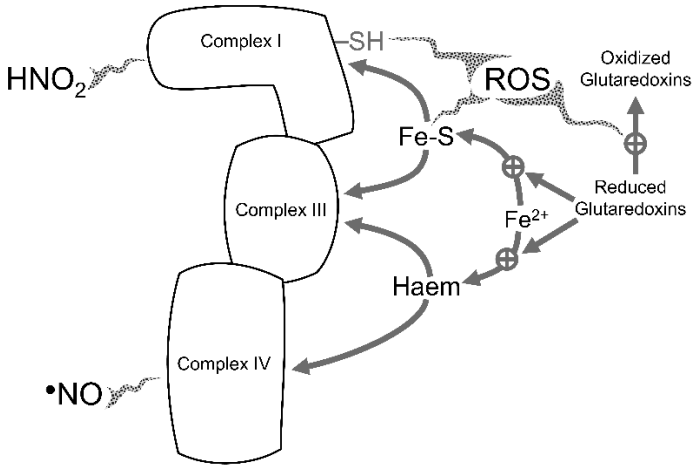

Fig. 4. Vulnerable parts of complexes of the canonical respiratory system: complex I has reactive thiol groups which can be oxidized, glutathionylated or nitrosated, complex I and complex III depend on Fe-S clusters, which, in turn, are sensitive to reactive oxygen species (ROS) and require glutaredoxin for their assembly, complexes III and IV contain different types of haem whose assembly is also dependent on glutaredoxins; additionally, complex IV can be inhibited by nitric oxide.

3.4.3. Regeneration

Another interesting trait in the majority of organisms possessing NDH2s and AOXs is their ability for regeneration. Indeed, mitochondria were found to be profoundly involved in the related phenomena of regeneration, cell proliferation, and stem-cell identity (Chen et al., 2009; Franco et al., 2013; Kasahara and Scorrano, 2014; Muliyil and Narasimha, 2014; Wanet et al., 2015). Regeneration in hydra is mainly morphallaxis, the re-differentiation of

existing stem cells with negligible proliferation (Bosch, 2007). There are several studies indicating that stem cells often rely on glycolysis, as they exhibit immature mitochondria, while differentiated cells get energy by respiration (Wanet et al., 2015; Slaninova et al., 2016). Here again, the transition from glycolysis to respiration, similar to changes occurring during the hypoxia/reoxygenation transition, may take place in stem cells during regeneration. In this case, the stem cells of animals containing NDH2s and AOXs, may have the ability to switch between glycolytic and respiratory metabolism more quickly compared to those of terrestrial vertebrates. While the assembly of a functional canonical RS would require the *de novo* synthesis of prosthetic groups (haem, Fe-S clusters) for RS complexes, the prosthetic group of NDH2, FAD, is synthesized in anaerobic cells, while AOX requires only iron. Thus during regeneration, NDH2 and AOX would enable oxidation of reducing equivalents formed in Krebs cycle in maturating mitochondria. Reports on the involvement of AOX in development, particularly in apoptosis and cellular differentiation, were recently published (Andjelković et al., 2016). It was found that ectopic expression of AOX from *Ciona intestinalis* may rescue developmental abnormalities in fruit flies by probable modulation of the c-Jun N-terminal kinase (JNK) signalling pathway (Andjelković et al., 2016). Thus, the consequences of the presence of AOX in the RS may spread beyond the reaction it catalyses. These effects may depend on AOX's influence on mitochondrial membrane potential (and signalling mediated by the membrane potential), ROS, calcium, and other messengers associated with mitochondria.

3.4.4. Testable predictions and notes in proof

We suggest that factors which would regularly compromise the operation of the canonical RS would also favour the retention of genes coding for alternative respiratory enzymes. Specifically, these factors are: regular changes in oxygen level (hypoxia followed by reoxygenation), frequent metabolic rearrangements associated with animal and plant stem cells, ROS attacks from the host immune system, lack of components necessary for synthesis of essential prosthetic groups such as haem or Fe-S clusters, and covalent chemical modifications to the components of canonical RS such as thiol oxidation and nitrosylation which lead to inhibition.

Modern cloning techniques allow for the introduction of genes coding for alternative RS enzymes in non-natural hosts. Thus, organisms which heterologously express alternative RS enzymes are expected to be resistant to conditions threatening proper operation of the canonical RS – hypoxia/reoxygenation, iron-chelating compounds, inhibitors of glutathione

and NADPH synthesis (necessary for Fe-S cluster assembly), oxidants, and NO-donors. On the other hand, those animals which are resistant these stress conditions may have a functional alternative RS. Several recent studies confirm our assumptions on the role of alternative RS enzymes in resistance to hypoxia/reoxygenation. For instance, the study of Liu and Guo (2017) shows that two isoenzymes of AOX are expressed in gill, mantle, gonads, adductor, and labial tissues of *C. virginica*. While isoenzyme A is expressed under physiological oxygen levels, expression of isoenzyme B, a result of alternative splicing, is triggered by prolonged air exposure. Pacific oyster *Crassostrea gigas*, which has both NDH2 and AOX, can rapidly adapt to changes in oxygen level (Sussarellu et al., 2013). In contrast, *Trichoplax adhaerens* was shown to be sensitive to fluctuations in water salinity (Pearse and Voigt, 2007), temperature, and acidity (Schleicherová et al., 2017), however, this animal has developed molecular machinery to resist hypoxia (Loenarz et al., 2011).

4. Alternative respiratory enzymes as therapeutic tools and metabolic modulators

4.1. By-passing defects in canonical RS complexes

Since the 2000s, studies investigating the heterologous expression of genes encoding AOXs and NDH2s have been performed in various model systems. It has been revealed that these enzymes can be satisfactory substitutes for impaired complexes of the canonical RS. The ability of AOX to rescue defects in complexes III or IV was studied using AOX from either the sea squirt *Ciona intestinalis* or the fungus *Emericella nidulans* (Perales-Clemente et al., 2008; Fernandez-Ayala et al., 2009; Schiff et al., 2012; El-Khoury et al., 2014). The ability of NDH2 to rescue complex I defects was exhaustively studied by using internal NDH2 Ndi1p from the budding yeast *Saccharomyces cerevisiae* (Seo et al., 2006a; Seo et al., 2006b; Yagi et al., 2006a; Yagi et al., 2006b; DeCorby et al., 2007; Marella et al., 2007; Sherer et al., 2007; Barber-Singh et al., 2009; Marella et al., 2009; Barber-Singh et al., 2010; Marella et al., 2010; Sanz et al., 2010; Cho et al., 2012; Vilain et al., 2012; Santidrian et al., 2013; Cossard et al., 2015). The rescuing of RS defects by both branching enzymes was observed in numerous models, namely the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, cultured human cells, mice, and rats. The approach of gene therapy using NDH2s and AOXs is rapidly developing with extension to pathological states indirectly connected with respiration. For instance, AOX from *Ciona intestinalis* showed rescuing of various pathological symptoms in fruit flies with modelled Alzheimer disease (El-Khoury et al., 2016). As mentioned above, AOX was surprisingly able to rescue developmental abnormalities related to thoracic closure in fruit fly (Andjelković et al., 2016). It is also

planned to apply AOX towards ischemia injuries (Rustin and Jacobs, 2009; Mills et al., 2016). Yeast Ndi1p also rescued organisms with a spectrum of mitochondria-related disorders, including Parkinson's disease, breast cancer, and optic neuropathy (Seo et al., 2006b; Marella et al., 2007; Sherer et al., 2007; Barber-Singh et al., 2009; Marella et al., 2009; Barber-Singh et al., 2010; Marella et al., 2010; Sanz et al., 2010; Vilain et al., 2012; Santidrian et al., 2013).

4.2. NDH2 as a tool to study ageing

Yeast Ndi1p prolonged the lifespan of model organisms, implying it may have broader therapeutic applications (Bahadorani et al., 2010; Sanz et al., 2010; Hur et al., 2013). Similar lifespan extension was conferred by Ndx, the NDH2 from *Ciona intestinalis* (Gospodaryov et al., 2014). It was initially assumed that lifespan extension by NDH2 is due to a) a decrease in NADH/NAD^+ ratio (Stefanatos and Sanz, 2011; Scialò et al., 2013; Hur et al., 2014) or b) a decrease in ROS production by complex I (Scialò et al., 2013). It was recently shown that mitochondria of Ndi1p-expressing cells produced more ROS (Scialò et al., 2016). It was suggested that Ndi1p increased the pool of reduced ubiquinone, therefore promoting reverse electron transport via complex I, and consequently ROS production (Scialò et al., 2016). With this in mind, another hypothesis of lifespan extension by NDH2 suggests that it is due to hormesis caused by excess ROS (Hur et al., 2014; Sanz, 2016). Both Ndi1p- and Ndx-expressing animals were found to be resistant to xenobiotics such as paraquat, menadione, alloxan, and other electrophilic compounds (Bahadorani et al., 2010; Gospodaryov et al., 2014; Gospodaryov et al., 2016). It suggests that the target of ROS-induced hormesis could be Nrf2 (nuclear factor erythroid-derived 2 factor 2), a transcription factor induced by ROS, redox-cycling, and/or electrophilic compounds (Lewis et al., 2010). Several recent studies have demonstrated that activation of Nrf2 may lead to lifespan extension. Particularly, relatively high levels of activated Nrf2 were found in the long-lived rodent the naked mole rat (Lewis et al., 2010; Lewis et al., 2015). Activation of Nrf2 by lithium treatment was also found to prolong lifespan in fruit fly (Castillo-Quan et al., 2016).

Previous and ongoing studies of the organisms which heterologously express enzymes of the alternative RS will help to discover the physiological roles of these proteins. In this case, in addition to their influence on bioenergetic processes, NDH2s and AOXs are modulators of cellular metabolism. They allow for the observation of how one extra reaction in the RS may cause global effects on other metabolic and signalling pathways.

5. Conclusions and perspectives

Many bacteria, and the mitochondria of many plants, fungi, protists, and some animals operate with a branched, non-canonical, RS. The branching means that the RS contains enzymes capable of passing electrons and protons from NADH to ubiquinone without proton pumping (NDH2s) and/or an enzyme capable of accepting electrons from ubiquinol and passing them on to oxygen (AOX). After their initial discovery, these enzymes were the research focus of a small number of comparative biochemists and biophysicists. NDH2s and AOXs have received renewed attention upon being recognized as convenient substitutes for impaired multi-protein complexes of the canonical RS. Enzymes of both types are being actively explored as gene therapy strategies to cure RS diseases in humans caused by mutations in either nuclear or mitochondrial DNA. It has been convincingly proven that NDH2 and AOX allow the survival of organisms with mutations in genes which code for subunits of RS complexes that are models for human diseases. AOX is also suggested to be of use in ischemic tissues, and both enzymes are being explored for the potential to cure several neurological diseases. Operating along with the canonical RS, NDH2s and AOXs can change the physiological state of an organism. NDH2 extends lifespan and increases resistance to xenobiotics while AOX may influence signalling pathways responsible for development. BothAOXs and NDH2s are also suitable tools for studying metabolism and roles of ROS in cells, since they are able to modulate intramitochondrial and intracellular levels of ROS. Finally, an alternative RS operates in a number of parasitic organisms and can be a target for treatment of the diseases caused by them. In addition to possible application of NDH2s and AOXs in medicine and medical technology, their natural functions are not well understood.

Despite the fact that alternative RSs are well studied in certain organisms, the spectrum of roles played by NDH2s and AOXs remains mysterious. These enzymes help plants to survive abiotic stresses (drought, temperature, excess light, hypoxia via over-watering) (Xu et al., 2011; Juntawong et al., 2014; Saha et al., 2016; Dahal & Vanlerberghe, 2017), confer metabolic plasticity to fungi (Finnegan et al., 2003; Li et al., 2011; Rogov et al., 2014), and increase the virulence of protists (Duarte and Tomas, 2014; Rogov et al., 2014; Yang et al., 2017).

The discovery of branched RSs in animals has generated new questions about the roles of NDH2s and AOXs. The AOX may be responsible for oxyconformity of marine animals and, therefore, their adaptation to climate change or water pollution. We propose a more universal explanation for the function of NDH2s and AOXs, stating that they allow acclimation to regular changes in oxygen levels in certain environments. They may also be essential in conditions when the synthesis of cofactors like haem and Fe-S clusters is compromised. Understanding of the natural function of alternative RS enzymes may provide clues about the evolution of the RS.

Acknowledgments

None to declare

Funding

D. P. is supported by Ministry of Education and Science of Ukraine (grant number 0115U002304). A.E.M. is supported by a Discovery Grant from the Natural Sciences and Engineering Research Council in Canada and the Canada Foundation for Innovation.

References

Abele, D., Philip, E., Gonzalez, P.M., Puntarulo, S., 2007. Marine invertebrate mitochondria and oxidative stress. Front. Biosci. 12, 933–946.

Albury, M.S., Elliott, C., Moore, A.L., 2010. Ubiquinol-binding site in the alternative oxidase: mutagenesis reveals features important for substrate binding and inhibition. Biochim. Biophys. Acta 1797, 1933–1939. doi:10.1016/j.bbabio.2010.01.013

Andjelković, A., Kemppainen, K.K., Jacobs, H.T., 2016. Ligand-bound GeneSwitch causes developmental aberrations in *Drosophila* that are alleviated by the alternative oxidase. G3 (Bethesda) 6, 2839–2846. doi:10.1534/g3.116.030882

Bahadorani, S., Cho, J., Lo, T., Contreras, H., Lawal, H.O., Krantz, D.E., Bradley, T.J., Walker, D.W., 2010. Neuronal expression of a single-subunit yeast NADH-ubiquinone oxidoreductase (Ndi1) extends *Drosophila* lifespan. Aging Cell 9, 191–202. doi:10.1111/j.1474-9726.2010.00546.x

Barber-Singh, J., Seo, B.B., Matsuno-Yagi, A., Yagi, T., 2010. Protective role of rAAV-NDI1, serotype 5, in an acute MPTP mouse Parkinson's model. Parkinsons Dis. 2011, 438370. doi:10.4061/2011/438370

Barber-Singh, J., Seo, B.B., Nakamaru-Ogiso, E., Lau, Y.-S., Matsuno-Yagi, A., Yagi, T., 2009. Neuroprotective effect of long-term NDI1 gene expression in a chronic mouse model of Parkinson disorder. Rejuvenation Res. 12, 259–267. doi:10.1089/rej.2009.0854

Berthold, D.A., Stenmark, P., 2003. Membrane-bound diiron carboxylate proteins. Annu. Rev. Plant Biol. 54, 497–517. doi:10.1146/annurev.arplant.54.031902.134915

Bleier, L., Wittig, I., Heide, H., Steger, M., Brandt, U., Dröse, S., 2015. Generator-specific targets of mitochondrial reactive oxygen species. Free Radic. Biol. Med. 78, 1–10. doi:10.1016/j.freeradbiomed.2014.10.511

Borisov, V.B., Gennis, R.B., Hemp, J., Verkhovsky, M.I., 2011. The cytochrome *bd* respiratory oxygen reductases. Biochim. Biophys. Acta 1807, 1398–1413. doi:10.1016/j.bbabio.2011.06.016

Bosch, T.C.G., 2007. Why polyps regenerate and we don't: towards a cellular and molecular framework for *Hydra* regeneration. Dev. Biol. 303, 421–433. doi:10.1016/j.ydbio.2006.12.012

Brooks, G.A., 2002. Lactate shuttles in nature. Biochem. Soc. Trans. 30, 258–264. doi: 10.1042/bst0300258

Brown, G.C., Borutaite, V., 2004. Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. Biochim. Biophys. Acta 1658, 44–49. doi:10.1016/j.bbabio.2004.03.016

Burwell, L.S., Nadtochiy, S.M., Tompkins, A.J., Young, S., Brookes, P.S., 2006. Direct evidence for S-nitrosation of mitochondrial complex I. Biochem. J. 394, 627–634. doi:10.1042/BJ20051435

Caito, S.W., Aschner, M., 2015. Mitochondrial redox dysfunction and environmental exposures. Antioxid. Redox Signal. 23, 578–595. doi:10.1089/ars.2015.6289

Castillo-Quan, J.I., Li, L., Kinghorn, K.J., Ivanov, D.K., Tain, L.S., Slack, C., Kerr, F., Nespital, T., Thornton, J., Hardy, J., Bjedov, I., Partridge, L., 2016. Lithium promotes longevity through GSK3/NRF2-dependent hormesis. Cell Rep 15, 638–650. doi:10.1016/j.celrep.2016.03.041

Chen, C., Liu, Y., Liu, Y., Zheng, P., 2009. The axis of mTOR-mitochondria-ROS and stemness of the hematopoietic stem cells. Cell Cycle 8, 1158–1160. doi:10.4161/cc.8.8.8139

Cho, J., Hur, J.H., Graniel, J., Benzer, S., Walker, D.W., 2012. Expression of yeast NDI1 rescues a *Drosophila* complex I assembly defect. PLoS ONE 7, e50644. doi:10.1371/journal.pone.0050644

Chouchani, E.T., Pell, V.R., James, A.M., Work, L.M., Saeb-Parsy, K., Frezza, C., Krieg, T., Murphy, M.P., 2016. A Unifying mechanism for mitochondrial superoxide production during ischemia-reperfusion injury. Cell Metab. 23, 254–263. doi:10.1016/j.cmet.2015.12.009

Collman, J.P., Dey, A., Decreau, R.A., Yang, Y., Hosseini, A., Solomon, E.I., Eberspacher, T.A., 2008. Interaction of nitric oxide with a functional model of cytochrome c oxidase. Proc. Natl. Acad. Sci. U.S.A. 105, 9892–9896. doi:10.1073/pnas.0804257105

Cossard, R., Esposito, M., Sellem, C.H., Pitayu, L., Vasnier, C., Delahodde, A., Dassa, E.P., 2015. *Caenorhabditis elegans* expressing the *Saccharomyces cerevisiae* NADH alternative dehydrogenase Ndi1p, as a tool to identify new genes involved in complex I related diseases. Front. Genet. 6, 206. doi:10.3389/fgene.2015.00206

Cui, Y., Zhao, S., Wu, Z., Dai, P., Zhou, B., 2012. Mitochondrial release of the NADH dehydrogenase Ndi1 induces apoptosis in yeast. Mol. Biol. Cell 23, 4373–4382. doi:10.1091/mbc.E12-04-0281

Dahal, K., Vanlerberghe, G.C., 2017. Alternative oxidase respiration maintains both mitochondrial and chloroplast function during drought. New Phytologist 213, 560–571. doi:10.1111/nph.14169

Dahm, C.C., Moore, K., Murphy, M.P., 2006. Persistent S-nitrosation of complex I and other mitochondrial membrane proteins by S-nitrosothiols but not nitric oxide or peroxynitrite: implications for the interaction of nitric oxide with mitochondria. J. Biol. Chem. 281, 10056–10065. doi:10.1074/jbc.M512203200

Dassa, E.P., Dufour, E., Goncalves, S., Jacobs, H.T., Rustin, P., 2009a. The alternative oxidase, a tool for compensating cytochrome c oxidase deficiency in human cells. Physiol. Plant. 137, 427–434. doi:10.1111/j.1399-3054.2009.01248.x

Dassa, E.P., Dufour, E., Gonçalves, S., Paupe, V., Hakkaart, G.A.J., Jacobs, H.T., Rustin, P., 2009b. Expression of the alternative oxidase complements cytochrome c oxidase deficiency in human cells. EMBO Mol. Med. 1, 30–36. doi:10.1002/emmm.200900001

DeCorby, A., Gásková, D., Sayles, L.C., Lemire, B.D., 2007. Expression of Ndi1p, an alternative NADH:ubiquinone oxidoreductase, increases mitochondrial membrane potential in a *C. elegans* model of mitochondrial disease. Biochim. Biophys. Acta 1767, 1157–1163. doi:10.1016/j.bbabio.2007.07.003

Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H.-O., Huey, R.B., 2015. Ecophysiology. Climate change tightens a metabolic constraint on marine habitats. Science 348, 1132–1135. doi:10.1126/science.aaa1605

Dröse, S., Stepanova, A., Galkin, A., 2016. Ischemic A/D transition of mitochondrial complex I and its role in ROS generation. Biochim. Biophys. Acta 1857, 946–957. doi:10.1016/j.bbabio.2015.12.013.

Duarte, M., Tomás, A.M., 2014. The mitochondrial complex I of trypanosomatids - an overview of current knowledge. J. Bioenerg. Biomembr. 46, 299–311. doi:10.1007/s10863-014-9556-x

Dunn, A.K., Karr, E.A., Wang, Y., Batton, A.R., Ruby, E.G., Stabb, E.V., 2010. The alternative oxidase (AOX) gene in *Vibrio fischeri* is controlled by NsrR and upregulated in response to nitric oxide. Mol. Microbiol. 77, 44–55. doi:10.1111/j.1365-2958.2010.07194.x

Dym, O., Eisenberg, D., 2001. Sequence-structure analysis of FAD-containing proteins. Protein Science 10, 1712–1728. doi:10.1110/ps.12801

Ekau, W., Auel, H., Pörtner, H.-O., Gilbert, D., 2010. Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). Biogeosciences 7, 1669–1699. doi:10.5194/bg-7-1669-2010

Elguindy, M.M., Nakamaru-Ogiso, E., 2015. Apoptosis-inducing factor (AIF) and its family member protein, AMID, are rotenone-sensitive NADH:ubiquinone oxidoreductases (NDH-2). J. Biol. Chem. 290, 20815–20826. doi:10.1074/jbc.M115.641498

El-Khoury, R., Kaulio, E., Lassila, K.A., Crowther, D.C., Jacobs, H.T., Rustin, P., 2016. Expression of the alternative oxidase mitigates beta-amyloid production and toxicity in model systems. Free Radic. Biol. Med. 96, 57–66. doi:10.1016/j.freeradbiomed.2016.04.006

El-Khoury, R., Kemppainen, K.K., Dufour, E., Szibor, M., Jacobs, H.T., Rustin, P., 2014. Engineering the alternative oxidase gene to better understand and counteract mitochondrial defects: state of the art and perspectives. Br. J. Pharmacol. 171, 2243–2249. doi:10.1111/bph.12570

Endo, T., Ueno, K., Yonezawa, K., Mineta, K., Hotta, K., Satou, Y., Yamada, L., Ogasawara, M., Takahashi, H., Nakajima, A., Nakachi, M., Nomura, M., Yaguchi, J., Sasakura, Y., Yamasaki, C., Sera, M., Yoshizawa, A.C., Imanishi, T., Taniguchi, H., Inaba, K., 2011. CIPRO 2.5: *Ciona intestinalis* protein database, a unique integrated repository of large-scale omics data, bioinformatic analyses and curated annotation, with user rating and reviewing functionality. Nucleic Acids Res. 39, D807-814. doi:10.1093/nar/gkq1144

Ercolesi, E., Tedeschi, G., Fiore, G., Negri, A., Maffioli, E., d'Ischia, M., Palumbo, A., 2012. Protein nitration as footprint of oxidative stress-related nitric oxide signaling pathways in developing *Ciona intestinalis*. Nitric Oxide 27, 18–24. doi:10.1016/j.niox.2012.03.012

Fernandez-Ayala, D.J.M., Sanz, A., Vartiainen, S., Kemppainen, K.K., Babusiak, M., Mustalahti, E., Costa, R., Tuomela, T., Zeviani, M., Chung, J., O'Dell, K.M.C., Rustin, P., Jacobs, H.T., 2009. Expression of the *Ciona intestinalis* alternative oxidase (AOX) in Drosophila complements defects in mitochondrial oxidative phosphorylation. Cell Metab. 9, 449–460. doi:10.1016/j.cmet.2009.03.004

Fernie, A.R., Carrari, F., Sweetlove, L.J., 2004. Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. Curr. Opin. Plant Biol. 7, 254–261. doi:10.1016/j.pbi.2004.03.007

Finnegan, P.M., Umbach, A.L., Wilce, J.A., 2003. Prokaryotic origins for the mitochondrial alternative oxidase and plastid terminal oxidase nuclear genes. FEBS Lett. 555, 425–430. doi:10.1016/S0014-5793(03)01309-7

Franco, C., Soares, R., Pires, E., Koci, K., Almeida, A.M., Santos, R., Coelho, A.V., 2013. Understanding regeneration through proteomics. Proteomics 13, 686–709. doi:10.1002/pmic.201200397

Gabaldón, T., Rainey, D., Huynen, M.A., 2005. Tracing the evolution of a large protein complex in the eukaryotes, NADH:ubiquinone oxidoreductase (Complex I). J. Mol. Biol. 348, 857–870. doi:10.1016/j.jmb.2005.02.067

Gospodaryov, D., Perkhulyn, N., Rovenko, B., Strilbytska, O., Semanyuk, U., Lushchak, O., 2016. Alternative NADH dehydrogenase from ascidian *Ciona intestinalis* prolongs lifespan of fruit fly and confers it resistance to inorganic and organic toxicants. Biochim. Biophys. Acta 1857, e38. doi:10.1016/j.bbabio.2016.04.115

Gospodaryov, D.V., Lushchak, O.V., Rovenko, B.M., Perkhulyn, N.V., Gerards, M., Tuomela, T., Jacobs, H.T., 2014. *Ciona intestinalis* NADH dehydrogenase NDX confers stress-resistance and extended lifespan on *Drosophila*. Biochim. Biophys. Acta 1837, 1861–1869. doi:10.1016/j.bbabio.2014.08.001

Handy, D.E., Loscalzo, J., 2012. Redox regulation of mitochondrial function. Antioxid. Redox Signal. 16, 1323–1367. doi:10.1089/ars.2011.4123

Heikal, A., Nakatani, Y., Dunn, E., Weimar, M.R., Day, C.L., Baker, E.N., Lott, J.S., Sazanov, L.A., Cook, G.M., 2014. Structure of the bacterial type II NADH dehydrogenase: a monotopic membrane protein with an essential role in energy generation. Mol. Microbiol. 91, 950–964. doi:10.1111/mmi.12507

Hildebrandt, T.M., Grieshaber, M.K., 2008. Redox regulation of mitochondrial sulfide oxidation in the lugworm, *Arenicola marina*. J. Exp. Biol. 211, 2617–2623. doi:10.1242/jeb.019729

Hur, J.H., Bahadorani, S., Graniel, J., Koehler, C.L., Ulgherait, M., Rera, M., Jones, D.L., Walker, D.W., 2013. Increased longevity mediated by yeast NDI1 expression in *Drosophila* intestinal stem and progenitor cells. Aging (Albany NY) 5, 662–681. doi:10.18632/aging.100595

Hur, J.H., Stork, D.A., Walker, D.W., 2014. Complex-I-ty in aging. J. Bioenerg. Biomembr. 46, 329–335. doi:10.1007/s10863-014-9553-0

Ito, K., Ogata, T., Kakizaki, Y., Elliott, C., Albury, M.S., Moore, A.L., 2011. Identification of a gene for pyruvate-insensitive mitochondrial alternative oxidase expressed in the thermogenic appendices in *Arum maculatum*. Plant Physiol. 157, 1721–1732. doi:10.1104/pp.111.186932

Iwata, M., Lee, Y., Yamashita, T., Yagi, T., Iwata, S., Cameron, A.D., Maher, M.J., 2012. The structure of the yeast NADH dehydrogenase (Ndi1) reveals overlapping binding sites for water- and lipid-soluble substrates. Proc. Natl. Acad. Sci. U.S.A. 109, 15247–15252. doi:10.1073/pnas.1210059109

Jastroch, M., Divakaruni, A.S., Mookerjee, S., Treberg, J.R., Brand, M.D., 2010. Mitochondrial proton and electron leaks. Essays Biochem. 47, 53–67. doi:10.1042/bse0470053

Juntawong, P., Sirikhachornkit, A., Pimjan, R., Sonthirod, C., Sangsrakru, D., Yoocha, T., Tangphatsornruang, S., Srinives, P., 2014. Elucidation of the molecular responses to waterlogging in *Jatropha* roots by transcriptome profiling. Front. Plant Sci. 5. doi:10.3389/fpls.2014.00658

Kalnenieks, U., Galinina, N., Strazdina, I., Kravale, Z., Pickford, J.L., Rutkis, R., Poole, R.K., 2008. NADH dehydrogenase deficiency results in low respiration rate and improved aerobic growth of *Zymomonas mobilis*. Microbiology 154, 989–994. doi:10.1099/mic.0.2007/012682-0

Kasahara, A., Scorrano, L., 2014. Mitochondria: from cell death executioners to regulators of cell differentiation. Trends Cell Biol. 24, 761–770. doi:10.1016/j.tcb.2014.08.005

Kerscher, S., Dröse, S., Zickermann, V., Brandt, U., 2008. The three families of respiratory NADH dehydrogenases. Results Probl. Cell Differ. 45, 185–222. doi:10.1007/400_2007_028

Kerscher, S.J., 2000. Diversity and origin of alternative NADH:ubiquinone oxidoreductases. Biochim. Biophys. Acta 1459, 274–283.

Kim, J.-S., He, L., Lemasters, J.J., 2003. Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem. Biophys. Res. Commun. 304, 463–470.

Lenaz, G., Genova, M.L., 2010. Structure and organization of mitochondrial respiratory complexes: A new understanding of an old subject. Antioxid. Redox Signal. 12, 961–1008. doi:10.1089/ars.2009.2704

Lewis, K.N., Mele, J., Hayes, J.D., Buffenstein, R., 2010. Nrf2, a guardian of healthspan and gatekeeper of species longevity. Integr. Comp. Biol. 50, 829–843. doi:10.1093/icb/icq034

Lewis, K.N., Wason, E., Edrey, Y.H., Kristan, D.M., Nevo, E., Buffenstein, R., 2015. Regulation of Nrf2 signaling and longevity in naturally long-lived rodents. Proc. Natl. Acad. Sci. U.S.A. 112, 3722–3727. doi:10.1073/pnas.1417566112

Li, Q., Bai, Z., O'Donnell, A., Harvey, L.M., Hoskisson, P.A., McNeil, B., 2011. Oxidative stress in fungal fermentation processes: the roles of alternative respiration. Biotechnology Letters 33, 457–467. doi:10.1007/s10529-010-0471-x

Li, W., Sun, L., Liang, Q., Wang, J., Mo, W., Zhou, B., 2006. Yeast AMID homologue Ndi1p displays respiration-restricted apoptotic activity and is involved in chronological aging. Mol. Biol. Cell 17, 1802–1811. doi:10.1091/mbc.E05-04-0333

Liu, M., Guo, X., 2017. A novel and stress adaptive alternative oxidase derived from alternative splicing of duplicated exon in oyster *Crassostrea virginica*. Sci Rep 7, 10785. doi:10.1038/s41598-017-10976-w

Loenarz, C., Coleman, M.L., Boleininger, A., Schierwater, B., Holland, P.W.H., Ratcliffe, P.J., Schofield, C.J., 2011. The hypoxia-inducible transcription factor pathway regulates oxygen sensing in the simplest animal, *Trichoplax adhaerens*. EMBO Rep. 12, 63–70. doi:10.1038/embor.2010.170

Loor, G., Kondapalli, J., Iwase, H., Chandel, N.S., Waypa, G.B., Guzy, R.D., Vanden Hoek, T.L., Schumacker, P.T., 2011. Mitochondrial oxidant stress triggers cell death in simulated ischemia-reperfusion. Biochim. Biophys. Acta 1813, 1382–1394. doi:10.1016/j.bbamcr.2010.12.008

Lushchak, V.I., 2011. Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 153, 175–190. doi:10.1016/j.cbpc.2010.10.004

Mailloux, R.J., Treberg, J.R., 2016. Protein S-glutathionlyation links energy metabolism to redox signaling in mitochondria. Redox Biol. 8, 110–118. doi:10.1016/j.redox.2015.12.010

Marcet-Houben, M., Marceddu, G., Gabaldón, T., 2009. Phylogenomics of the oxidative phosphorylation in fungi reveals extensive gene duplication followed by functional divergence. BMC Evol. Biol. 9, 295. doi:10.1186/1471-2148-9-295

Marella, M., Seo, B.B., Matsuno-Yagi, A., Yagi, T., 2007. Mechanism of cell death caused by complex I defects in a rat dopaminergic cell line. J. Biol. Chem. 282, 24146–24156. doi:10.1074/jbc.M701819200

Marella, M., Seo, B.B., Thomas, B.B., Matsuno-Yagi, A., Yagi, T., 2010. Successful amelioration of mitochondrial optic neuropathy using the yeast NDI1 gene in a rat animal model. PLoS ONE 5, e11472. doi:10.1371/journal.pone.0011472

Marella, M., Seo, B.B., Yagi, T., Matsuno-Yagi, A., 2009. Parkinson's disease and mitochondrial complex I: a perspective on the Ndi1 therapy. J. Bioenerg. Biomembr. 41, 493–497. doi:10.1007/s10863-009-9249-z

Marreiros, B.C., Sena, F.V., Sousa, F.M., Batista, A.P., Pereira, M.M., 2016. Type II NADH:quinone oxidoreductase family: Phylogenetic distribution, structural diversity and evolutionary divergences. Environ. Microbiol. doi:10.1111/1462-2920.13352

Matus-Ortega, M.G., Cárdenas-Monroy, C.A., Flores-Herrera, O., Mendoza-Hernández, G., Miranda, M., González-Pedrajo, B., Vázquez-Meza, H., Pardo, J.P., 2015. New complexes containing the internal alternative NADH dehydrogenase (Ndi1) in mitochondria of *Saccharomyces cerevisiae*: Mitochondrial complexes containing the alternative NADH dehydrogenase. Yeast 32, 629–641. doi:10.1002/yea.3086

Matus-Ortega, M.G., Salmerón-Santiago, K.G., Flores-Herrera, O., Guerra-Sánchez, G., Martínez, F., Rendón, J.L., Pardo, J.P., 2011. The alternative NADH dehydrogenase is present in mitochondria of some animal taxa. Comp. Biochem. Physiol. Part D Genomics Proteomics 6, 256–263. doi:10.1016/j.cbd.2011.05.002

McDonald, A., Vanlerberghe, G., 2004. Branched mitochondrial electron transport in the *Animalia*: presence of alternative oxidase in several animal phyla. IUBMB Life 56, 333–341. doi:10.1080/1521-6540400000876

McDonald, A.E., Vanlerberghe, G.C., Staples, J.F., 2009. Alternative oxidase in animals: unique characteristics and taxonomic distribution. J. Exp. Biol. 212, 2627–2634. doi:10.1242/jeb.032151

McDonald, A.E., Vanlerberghe, G.C., 2006. Origins, evolutionary history, and taxonomic distribution of alternative oxidase and plastoquinol terminal oxidase. Comp. Biochem. Physiol. Part D Genomics Proteomics 1, 357–364. doi:10.1016/j.cbd.2006.08.001

Melo, A.M.P., Bandeiras, T.M., Teixeira, M., 2004. New insights into type II NAD(P)H:quinone oxidoreductases. Microbiol. Mol. Biol. Rev. 68, 603–616. doi:10.1128/MMBR.68.4.603-616.2004

Mills, E.L., Kelly, B., Logan, A., Costa, A.S.H., Varma, M., Bryant, C.E., Tourlomousis, P., Däbritz, J.H.M., Gottlieb, E., Latorre, I., Corr, S.C., McManus, G., Ryan, D., Jacobs, H.T., Szibor, M., Xavier, R.J., Braun, T., Frezza, C., Murphy, M.P., O'Neill, L.A., 2016. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 167, 457–470.e13. doi:10.1016/j.cell.2016.08.064

Muliyil, S., Narasimha, M., 2014. Mitochondrial ROS regulates cytoskeletal and mitochondrial remodeling to tune cell and tissue dynamics in a model for wound healing. Dev. Cell 28, 239–252. doi:10.1016/j.devcel.2013.12.019

Murphy, M.P., 2009. How mitochondria produce reactive oxygen species. Biochem. J. 417, 1–13. doi:10.1042/BJ20081386

Ojha, S., Meng, E.C., Babbitt, P.C., 2007. Evolution of function in the "two dinucleotide binding domains" flavoproteins. PLoS Comput. Biol. 3, e121. doi:10.1371/journal.pcbi.0030121

Onda, Y., Kato, Y., Abe, Y., Ito, T., Morohashi, M., Ito, Y., Ichikawa, M., Matsukawa, K., Kakizaki, Y., Koiwa, H., Ito, K., 2008. Functional coexpression of the mitochondrial alternative oxidase and uncoupling protein underlies thermoregulation in the thermogenic florets of skunk cabbage. Plant Physiol. 146, 636–645. doi:10.1104/pp.107.113563

Pearse, V.B., Voigt, O., 2007. Field biology of placozoans (*Trichoplax*): distribution, diversity, biotic interactions. Integr. Comp. Biol. 47, 677–692. doi:10.1093/icb/icm015

Perales-Clemente, E., Bayona-Bafaluy, M.P., Pérez-Martos, A., Barrientos, A., Fernández-Silva, P., Enriquez, J.A., 2008. Restoration of electron transport without proton pumping in mammalian mitochondria. Proc. Natl. Acad. Sci. U.S.A. 105, 18735–18739. doi:10.1073/pnas.0810518105

Pichaud, N., Rioux, P., Blier, P.U., 2012. *In situ* quantification of mitochondrial respiration in permeabilized fibers of a marine invertebrate with low aerobic capacity. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 161, 429–435. doi:10.1016/j.cbpa.2012.01.001

Picklo, M.J., Long, E.K., Vomhof-DeKrey, E.E., 2015. Glutathionyl systems and metabolic dysfunction in obesity. Nutr. Rev. 73, 858–868. doi:10.1093/nutrit/nuv042

Poole, R.K., Cook, G.M., 2000. Redundancy of aerobic respiratory chains in bacteria? Routes, reasons and regulation, in: Advances in Microbial Physiology. Elsevier, pp. 165–224.

Pun, P.B.L., Logan, A., Darley-Usmar, V., Chacko, B., Johnson, M.S., Huang, G.W., Rogatti, S., Prime, T.A., Methner, C., Krieg, T., Fearnley, I.M., Larsen, L., Larsen, D.S., Menger, K.E., Collins, Y., James, A.M., Kumar, G.D.K., Hartley, R.C., Smith, R.A.J., Murphy, M.P., 2014. A mitochondria-targeted mass spectrometry probe to detect glyoxals: implications for diabetes. Free Radic. Biol. Med. 67, 437–450. doi:10.1016/j.freeradbiomed.2013.11.025

Pun, P.B.L., Murphy, M.P., 2012. Pathological significance of mitochondrial glycation. Int. J. Cell Biol. 2012, 843505. doi:10.1155/2012/843505

Rabbani, N., Thornalley, P.J., 2008. Dicarbonyls linked to damage in the powerhouse: glycation of mitochondrial proteins and oxidative stress. Biochem. Soc. Trans. 36, 1045–1050. doi:10.1042/BST0361045

Rasmusson, A.G., Soole, K.L., Elthon, T.E., 2004. Alternative NAD(P)H dehydrogenases of plant mitochondria. Annu. Rev. Plant Biol. 55, 23–39. doi:10.1146/annurev.arplant.55.031903.141720

Rivera-Ingraham, G.A., Rocchetta, I., Meyer, S., Abele, D., 2013. Oxygen radical formation in anoxic transgression and anoxia-reoxygenation: foe or phantom? Experiments with a hypoxia tolerant bivalve. Mar. Environ. Res. 92, 110–119. doi:10.1016/j.marenvres.2013.09.007

Robertson, A., Schaltz, K., Neimanis, K., Staples, J.F., McDonald, A.E., 2016. Heterologous expression of the *Crassostrea gigas* (Pacific oyster) alternative oxidase in the yeast Saccharomyces cerevisiae. J. Bioenerg. Biomembr. 48, 509–520. doi:10.1007/s10863-016-9685-5

Rogov, A.G., Sukhanova, E.I., Uralskaya, L.A., Aliverdieva, D.A., Zvyagilskaya, R.A., 2014. Alternative oxidase: distribution, induction, properties, structure, regulation, and functions. Biochemistry (Mosc.) 79, 1615–1634. doi:10.1134/S0006297914130112

Rustin, P., Jacobs, H.T., 2009. Respiratory chain alternative enzymes as tools to better understand and counteract respiratory chain deficiencies in human cells and animals. Physiol. Plant. 137, 362–370. doi:10.1111/j.1399-3054.2009.01249.x

Ryan, J.F., Pang, K., Schnitzler, C.E., Nguyen, A.-D., Moreland, R.T., Simmons, D.K., Koch, B.J., Francis, W.R., Havlak, P., NISC Comparative Sequencing Program, Smith, S.A., Putnam, N.H., Haddock, S.H.D., Dunn, C.W., Wolfsberg, T.G., Mullikin, J.C., Martindale, M.Q., Baxevanis, A.D., 2013. The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. Science 342, 1242592. doi:10.1126/science.1242592

Saha, B., Borovskii, G., Panda, S.K., 2016. Alternative oxidase and plant stress tolerance. Plant Signal. Behav. 11, e1256530. doi:10.1080/15592324.2016.1256530

Santidrian, A.F., Matsuno-Yagi, A., Ritland, M., Seo, B.B., LeBoeuf, S.E., Gay, L.J., Yagi, T., Felding-Habermann, B., 2013. Mitochondrial complex I activity and NAD^+/NADH balance regulate breast cancer progression. J. Clin. Invest. 123, 1068–1081. doi:10.1172/JCI64264

Sanz, A., 2016. Mitochondrial reactive oxygen species: Do they extend or shorten animal lifespan? Biochim. Biophys. Acta 1857, 1116–1126. doi:10.1016/j.bbabio.2016.03.018

Sanz, A., Soikkeli, M., Portero-Otín, M., Wilson, A., Kemppainen, E., McIlroy, G., Ellilä, S., Kemppainen, K.K., Tuomela, T., Lakanmaa, M., Kiviranta, E., Stefanatos, R., Dufour, E., Hutz, B., Naudí, A., Jové, M., Zeb, A., Vartiainen, S., Matsuno-Yagi, A., Yagi, T., Rustin, P., Pamplona, R., Jacobs, H.T., 2010. Expression of the yeast NADH dehydrogenase Ndi1 in *Drosophila* confers increased lifespan independently of dietary restriction. Proc. Natl. Acad. Sci. U.S.A. 107, 9105–9110. doi:10.1073/pnas.0911539107

Sarti, P., Forte, E., Mastronicola, D., Giuffrè, A., Arese, M., 2012. Cytochrome c oxidase and nitric oxide in action: molecular mechanisms and pathophysiological implications. Biochim. Biophys. Acta 1817, 610–619. doi:10.1016/j.bbabio.2011.09.002

Schiff, M., Bénit, P., Jacobs, H.T., Vockley, J., Rustin, P., 2012. Therapies in inborn errors of oxidative metabolism. Trends Endocrinol. Metab. 23, 488–495. doi:10.1016/j.tem.2012.04.006

Schleicherová, D., Dulias, K., Osigus, H.-J., Paknia, O., Hadrys, H., Schierwater, B., 2017. The most primitive metazoan animals, the placozoans, show high sensitivity to increasing ocean temperatures and acidities. Ecol Evol 7, 895–904. doi:10.1002/ece3.2678

Scialò, F., Mallikarjun, V., Stefanatos, R., Sanz, A., 2013. Regulation of lifespan by the mitochondrial electron transport chain: reactive oxygen species-dependent and reactive oxygen species-independent mechanisms. Antioxid. Redox Signal. 19, 1953–1969. doi:10.1089/ars.2012.4900

Scialò, F., Sriram, A., Fernández-Ayala, D., Gubina, N., Lõhmus, M., Nelson, G., Logan, A., Cooper, H.M., Navas, P., Enríquez, J.A., Murphy, M.P., Sanz, A., 2016. Mitochondrial ROS Produced via Reverse Electron Transport Extend Animal Lifespan. Cell Metab. 23, 725–734. doi:10.1016/j.cmet.2016.03.009

Seo, B.B., Marella, M., Yagi, T., Matsuno-Yagi, A., 2006a. The single subunit NADH dehydrogenase reduces generation of reactive oxygen species from complex I. FEBS Lett. 580, 6105–6108. doi:10.1016/j.febslet.2006.10.008

Seo, B.B., Nakamaru-Ogiso, E., Flotte, T.R., Matsuno-Yagi, A., Yagi, T., 2006b. In vivo complementation of complex I by the yeast Ndi1 enzyme. Possible application for treatment of Parkinson disease. J. Biol. Chem. 281, 14250–14255. doi:10.1074/jbc.M600922200

Sherer, T.B., Richardson, J.R., Testa, C.M., Seo, B.B., Panov, A.V., Yagi, T., Matsuno-Yagi, A., Miller, G.W., Greenamyre, J.T., 2007. Mechanism of toxicity of pesticides acting at complex I: relevance to environmental etiologies of Parkinson's disease. J. Neurochem. 100, 1469–1479. doi:10.1111/j.1471-4159.2006.04333.x

Shiva, S., 2010. Mitochondria as metabolizers and targets of nitrite. Nitric Oxide 22, 64–74. doi:10.1016/j.niox.2009.09.002

Slaninova, V., Krafcikova, M., Perez-Gomez, R., Steffal, P., Trantirek, L., Bray, S.J., Krejci, A., 2016. Notch stimulates growth by direct regulation of genes involved in the control of glycolysis and the tricarboxylic acid cycle. Open Biol. 6, 150155. doi:10.1098/rsob.150155

Stefanatos, R., Sanz, A., 2011. Mitochondrial complex I: a central regulator of the aging process. Cell Cycle 10, 1528–1532. doi:10.4161/cc.10.10.15496

Stehling, O., Lill, R., 2013. The role of mitochondria in cellular iron-sulfur protein biogenesis: mechanisms, connected processes, and diseases. Cold Spring Harb. Perspect. Biol. 5, a011312. doi:10.1101/cshperspect.a011312

Storey, K.B. (Ed.), 2004. Functional metabolism: regulation and adaptation. John Wiley & Sons, Hoboken, N.J.

Sussarellu, R., Dudognon, T., Fabioux, C., Soudant, P., Moraga, D., Kraffe, E., 2013. Rapid mitochondrial adjustments in response to short-term hypoxia and re-oxygenation in the Pacific oyster, *Crassostrea gigas*. J. Exp. Biol. 216, 1561–1569. doi:10.1242/jeb.075879

Sweetlove, L.J., Beard, K.F.M., Nunes-Nesi, A., Fernie, A.R., Ratcliffe, R.G., 2010. Not just a circle: flux modes in the plant TCA cycle. Trends Plant Sci. 15, 462–470. doi:10.1016/j.tplants.2010.05.006

Szal, B., Jolivet, Y., Hasenfratz-Sauder, M.-P., Dizengremel, P., Rychter, A.M., 2003. Oxygen concentration regulates alternative oxidase expression in barley roots during hypoxia and post-hypoxia. Physiologia Plantarum 119, 494–502. doi:10.1046/j.1399-3054.2003.00161.x

Taylor, E.R., Hurrell, F., Shannon, R.J., Lin, T.-K., Hirst, J., Murphy, M.P., 2003. Reversible glutathionylation of complex I increases mitochondrial superoxide formation. J. Biol. Chem. 278, 19603–19610. doi:10.1074/jbc.M209359200

Torre, L., Abele, D., Lagger, C., Momo, F., Sahade, R., 2014. When shape matters: strategies of different Antarctic ascidians morphotypes to deal with sedimentation. Mar. Environ. Res. 99, 179–187. doi:10.1016/j.marenvres.2014.05.014

Tostevin, R., Wood, R.A., Shields, G.A., Poulton, S.W., Guilbaud, R., Bowyer, F., Penny, A.M., He, T., Curtis, A., Hoffmann, K.H., Clarkson, M.O., 2016. Low-oxygen waters limited habitable space for early animals. Nat Commun 7, 12818. doi:10.1038/ncomms12818

Vanlerberghe, G.C., Martyn, G.D., Dahal, K., 2016. Alternative oxidase: a respiratory electron transport chain pathway essential for maintaining photosynthetic performance during drought stress. Physiol. Plant. 157, 322–337. doi:10.1111/ppl.12451

Vaquer-Sunyer, R., Duarte, C.M., 2008. Thresholds of hypoxia for marine biodiversity. Proc. Natl. Acad. Sci. U.S.A. 105, 15452–15457. doi:10.1073/pnas.0803833105

Vilain, S., Esposito, G., Haddad, D., Schaap, O., Dobreva, M.P., Vos, M., Van Meensel, S., Morais, V.A., De Strooper, B., Verstreken, P., 2012. The yeast complex I equivalent NADH dehydrogenase rescues pink1 mutants. PLoS Genet. 8, e1002456. doi:10.1371/journal.pgen.1002456

Wanet, A., Arnould, T., Najimi, M., Renard, P., 2015. Connecting Mitochondria, metabolism, and stem cell fate. Stem Cells Dev. 24, 1957–1971. doi:10.1089/scd.2015.0117

Watling, J.R., Robinson, S.A., Seymour, R.S., 2006. Contribution of the alternative pathway to respiration during thermogenesis in flowers of the sacred lotus. Plant Physiol. 140, 1367–1373. doi:10.1104/pp.105.075523

Xu, F., Yuan, S., Lin, H.-H., 2011. Response of mitochondrial alternative oxidase (AOX) to light signals. Plant Signal. Behav. 6, 55–58. doi:10.4161/psb.6.1.14192

Yagi, T., Seo, B.B., Nakamaru-Ogiso, E., Marella, M., Barber-Singh, J., Yamashita, T., Kao, M.-C., Matsuno-Yagi, A., 2006a. Can a single subunit yeast NADH dehydrogenase (Ndi1) remedy diseases caused by respiratory complex I defects? Rejuvenation Res. 9, 191–197. doi:10.1089/rej.2006.9.191

Yagi, T., Seo, B.B., Nakamaru-Ogiso, E., Marella, M., Barber-Singh, J., Yamashita, T., Matsuno-Yagi, A., 2006b. Possibility of transkingdom gene therapy for complex I diseases. Biochim. Biophys. Acta 1757, 708–714. doi:10.1016/j.bbabio.2006.01.011

Yang, Y., Yu, Y., Li, X., Li, J., Wu, Y., Yu, J., Ge, J., Huang, Z., Jiang, L., Rao, Y., Yang, M., 2017. Target elucidation by cocrystal structures of NADH-ubiquinone oxidoreductase of *Plasmodium falciparum* (*Pf* NDH2) with small molecule to eliminate drug-resistant malaria. J. Med. Chem. 60, 1994–2005, doi:10.1021/acs.jmedchem.6b01733

Young, L., May, B., Shiba, T., Harada, S., Inaoka, D.K., Kita, K., Moore, A.L., 2016. Structure and mechanism of action of the alternative quinol oxidases, in: Cramer, W.A., Kallas, T. (Eds.), Cytochrome Complexes: Evolution, Structures, Energy Transduction, and Signaling. Springer Netherlands, Dordrecht, pp. 375–394.

Table 1

Taxonomic distribution of AOX in animals using the NCBI classification scheme. The number of species containing a putative AOX sequence is listed for each group.

Placozoa		1
Porifera	Demospongiae	4
Eumetozoa		
Cnidaria	Anthozoa	15
	Hydrozoa	4
Ctenophora	Tentaculata	1
Bilateria		
Platyhelminthes	Rhabditophora	1
Protostomia		
Ecdysozoa		
Nematoda	Chromadorea	7
Panarthropoda		
Arthropoda	Chelicerata	3
	Hexapoda	11
	Crustacea	23
Tardigrada	Eutardigrada	3
Scalidophora	Priapulida	1
Lophotrochozoa		
Annelida	Polychaeta	7
Brachiopoda	Linguliformea	1
	Phoroniformea	1
Mollusca	Bivalvia	14
	Gastropoda	20
Rotifera	Bdelloidea	6
Deuterostomia		
Chordata		
Cephalochordata	Branchiostomidae	3
Tunicata	Ascidiacea	4
Echinodermata	Asterozoa	14
	Crinoidea	1
	Echinozoa	11
Hemichordata	Enteropneusta	1

Highlights

Type 2 NDH dehydrogenases (NDH2s) and alternative oxidases (AOXs) are relics of a metabolic arrangement characteristic for anaerobic and early aerobic organisms.

The physiological and bioenergetic roles of NDH2s and AOXs diversified during evolution, providing stress resistance, adaptation to certain environments, and thermogenesis.

Canonical respiratory system complexes can be substituted by NDH2s and AOXs during environmental changes which compromise the functioning of these enzymes.