

# Physiological Role of Alternative Oxidase (from Yeasts to Plants)

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**Abstract**—Mitochondria of all so far studied organisms, with the exception of Archaea, mammals, some yeasts, and protists, contain, along with the classical phosphorylating cytochrome pathway, a so-called cyanide-insensitive alternative oxidase (AOX) localized on the matrix side of the mitochondrial inner membrane, and electron transport through which is not coupled with ATP synthesis and energy accumulation. Mechanisms underlying plentiful functions of AOX in organisms at various levels of organization ranging from yeasts to plants are considered. First and foremost, AOX provides a chance of cell survival after inhibiting the terminal components of the main respiratory chain or losing the ability to synthesize these components. The vitally important role of AOX is obvious in thermogenesis of thermogenic plant organs where it becomes the only terminal oxidase with a very high activity, and the energy of substrate oxidation by this respiratory pathway is converted into heat, thus promoting evaporation of volatile substances attracting pollinating insects. AOX plays a fundamentally significant role in alleviating or preventing oxidative stress, thus ensuring the defense against a wide range of stresses and adverse environmental conditions, such as changes in temperature and light intensities, osmotic stress, drought, and attack by incompatible strains of bacterial pathogens, phytopathogens, or their elicitors. Participation of AOX in pathogen survival during its existence inside the host, in antiviral defense, as well as in metabolic rearrangements in plants during embryogenesis and cell differentiation is described. Examples are given to demonstrate that AOX might be an important tool to overcome the adverse aftereffects of restricted activity of the main respiratory chain in cells and whole animals.

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Mitochondria of many organisms including all plants, most fungi, algae, and some protists examined to date contain, in addition to the canonical cyanide-sensitive cytochrome oxidase of the respiratory chain, a cyanide-insensitive, hydroxamic acid-sensitive terminal oxidase called “alternative oxidase” (AOX). The AOX is an integral protein (32–36 kDa) of the inner mitochondrial membrane. It is encoded by the nuclear genome, is localized on the matrix side of the mitochondrial inner membrane, and is known to catalyze the four-electron oxidation of ubiquinol (reduced form of ubiquinone) by oxygen to water [1–3]. Electron transport through AOX is not coupled with ATP synthesis and energy conservation; thus, the energy of ubiquinol oxidation by oxygen is released as heat [1, 4–6].

In several yeast species with aerobic metabolism, active AOX was present at all growth stages [1]. In contrast, in the yeasts *Dipodascus (Endomyces) magnusii* [1] and some other organisms under normal conditions (i.e. under non-stress functioning of the main phosphorylat-

ing respiratory chain), the AOX activity was either low or indiscernible. However, the AOX activity (in yeasts, for example) increased significantly when the terminal part of the main respiratory chain was inhibited by the following treatments: a) growing the organisms on media deficient in the content of iron ions, sulfur, or copper; b) growing or incubation of resting cells in the presence of antimycin A, cyanide, or azide; c) growing or incubation of resting cells treated with inhibitors of mitochondrial transcription and translation; d) mutational changes in the nuclear or mitochondrial genomes; e) inhibition of the oxidative phosphorylation system; f) lowering of oxygen concentration [1]. Such stimulation of AOX activity, as deduced from the increasing amounts of AOX transcripts or AOX protein and from activation of a cyanide-resistant hydroxamate-sensitive oxidative pathway, was demonstrated not only with yeasts [1, 7–10] and other fungi [11–14], but also with algae [15], plants [16], protists [17], and *Drosophila* [18].

The appearance of cyanide- and antimycin A-insensitive respiration in mitochondria can also arise from changes in the physiological condition of the tissue, organ, or organism. A classical example is the manifold avalanche-like enhancement of AOX activity in thermogenic

*Abbreviations:* AOX, alternative oxidase; ROS, reactive oxygen species.

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tissues of aroid plants over a few days [19–21]. In this case, AOX becomes the only terminal oxidase, and heat release upon oxidation of substrates in the respiratory chain is sufficient to produce volatiles that attract pollinating insects. Another prominent example is the different degrees of AOX expression in trypanosomes at various stages of the organism's development. Mitochondria of the bloodstream form are devoid of cytochromes, and respiration is carried out exclusively by AOX [22], while in the insect form, having the fully competent respiratory chain, stability of the AOX transcript is diminished [22]. In the yeasts *Yarrowia lipolytica* (formerly *Candida lipolytica* and *Schizosaccharomyces lipolytica*) and *Pichia membranifaciens* grown on glucose, AOX is induced upon transition of the culture to the phase of stationary growth; in this case, AOX operates simultaneously with the cytochrome part of the respiratory chain [23–25]. In the yeast *P. pastoris*, AOX activity increased monotonously during culture growth and fell sharply upon depletion of glucose in the growth medium [2]. In mitochondria of the fungus *Metarhizium anisopliae*, the highest AOX activity was observed at the beginning and at the end of the fungal developmental cycle, during germination of aerial conidia and upon the formation of submerged conidia [26]. In the dimorphic fungus *Paracoccidioides brasiliensis* causing paracoccidioidomycosis in humans, the expression of an AOX-encoding gene increased significantly during conidia germination and formation of the yeast form [27, 28]. In the hemibiotrophic fungus *M. pernicioso*, a tropical pathogen causing a disease of cocoa, which first colonizes the living host tissues (biotrophic phase) and then grows on the dead plant (necrotic phase), the largest number of AOX transcripts was observed in the biotrophic phase [29]. Aging of potato slices [30] and fruit ripening [31, 32] were also shown to be accompanied by a significant activation of AOX.

In yeasts [33, 34] and plants [35–40], the level of AOX expression depended on availability of nutrients and the carbon source.

In fungi including yeasts, the AOX activity or the content of the AOX transcript increased substantially upon mild heat shock [41], as well as under conditions of oxidative [9, 10, 19, 28, 41–43] and osmotic [41] stresses. In pathogenic fungi, AOX activity increased sharply under the action of antifungal drugs [44].

In plants, depending on the tissue or organ, developmental phase, and metabolic status [3, 45–47], the expression of AOX was significantly increased in response to a wide range of stresses and adverse environmental conditions, such as changes in temperature and light intensity [47–53], osmotic stress [54–56], drought [54, 57], oxidative stress [58, 59], attack by incompatible strains of bacterial pathogens, phytopathogens or their elicitors [60–64], treatment with ethylene [65] or NO [65], and the addition of salicylic acid [66–68].

The role of the alternative respiratory pathway, unrelated to energy storage, has attracted attention of

researchers for a few decades. Several hypotheses have been proposed as outlined below.

The physiological role of AOX in thermogenesis of thermogenic plants is quite obvious. In thermogenic flowering organs, mitochondria undergo fundamental rearrangements of the respiratory chain: AOX becomes the only terminal oxidase with very high activity. The energy of substrate oxidation by this respiratory chain is converted into heat, which promotes evaporation of volatile substances, thus attracting pollinating insects [19, 20].

The role of AOX upon inhibiting the terminal components of the main respiratory chain or in losing the ability to synthesize these components is also quite conceivable. Operation of the alternative pathway under these conditions enables the reoxidation of cytoplasmic NADH, thus increasing the chances of cell survival. In addition, the phosphorylating activity at the first coupling site and the substrate phosphorylation are retained, while AOX ensures high oxidative activity required for biosynthetic processes in mitochondria. This situation occurs also in *Trypanosoma brucei*, the causative agent of sleeping sickness. At a certain stage of parasite development (the bloodstream form), AOX is the only terminal oxidase ensuring respiration and survival of the pathogen in host cells [22]. Similar events were observed in *Philasterides dicentrarchi*, causing the disease in turbot. Under normal oxygenation, respiration of the pathogen was sensitive to inhibition of the cytochrome chain by antimycin A, whereas under hypoxic conditions cyanide- and antimycin A-resistant respiration was induced [69].

The fundamental significance of AOX in alleviating or preventing oxidative stress is also evident. Recently, it has been shown that the *Aox1* promoter is sensitive to hydrogen peroxide [70]. Decrease in the reduction state of coenzyme Q, the donor of reducing equivalents for AOX, diminishes the production of superoxide anion radical and, eventually, production of hydrogen peroxide, the most stable reactive oxygen species. Such data have been obtained for yeasts [2, 9, 24, 26, 71, 72], other fungi [28, 41–43, 73–76], algae [15], and the plant *Arabidopsis thaliana* [77, 78]. Owing to its involvement in suppression of oxidative stress, AOX can decrease the threshold for the induction of programmed cell death [79].

In this connection, the role of AOX in response to drought, osmotic and oxidative stresses, as well as to treatment with toxins, elicitors, and pathogens is becoming clear.

Drought and osmotic stress are known to be accompanied by oxidative stress. Salt-stressed *A. thaliana* plants were marked by an increase in intracellular Na<sup>+</sup> content and elevated reactive oxygen species (ROS) production. In these plants, the expression of the *AtAox1a* gene was sharply increased [55]. The high AOX activity in roots and shoots provides *A. thaliana* plants with the ability to grow on media with high NaCl concentrations and to maintain intracellular Na<sup>+</sup> at low levels [55]. The involvement of

AOX in response to salt stress was also shown for *Medicago truncatula* [80]. In experiments with the alga *Chlamydomonas reinhardtii* grown in nitrate-rich media, participation of AOX in nitrate and nitrite reduction was demonstrated [81].

Elicitors and toxins of plant pathogens facilitate mitochondrial ROS formation [82]. Infection by pathogens and viruses leads to the accumulation of hydrogen peroxide, NO, ethylene, salicylic acid, and the methyl ester of jasmonic acids, which act as signaling molecules for the induction of a protective response of plants and induce the expression of the gene coding AOX or elevate the content of the plant protein [83]. All these compounds inhibit cytochrome-dependent electron transport in the respiratory chain. Infection by pathogens also activates the pentose phosphate pathway and NADP-dependent malic enzyme, which in turn increases the pools of NADPH and pyruvate acting as AOX activator in plants. *Nicotiana tabacum* mutants devoid of AOX were less tolerant to stresses caused by attacks of pathogenic bacteria, fungi, and sucking insects. Protective metabolites were less abundant in mutant plants than in the wild-type plants, whereas the ROS content and the percentage of cell death were higher than in the wild type [62, 84]. Thus, AOX in plants might represent a universal mechanism of response to oxidative stress [85] and stress conditions in general [62].

The role of AOX in the response to light stress is also becoming clear [48]. In photosynthesizing organisms, chloroplasts transform light energy into reducing equivalents. Since CO<sub>2</sub> fixation consumes only about 50% of the absorbed light energy, it is obvious that photosynthesis produces an excess of reducing equivalents. In the absence of energy dissipation, the excess of reducing equivalents would cause oxidative stress and damage to the photosynthetic apparatus. External stress factors inhibit CO<sub>2</sub> fixation, thus promoting the accumulation of excess ROS in chloroplasts. Obviously, mechanisms are needed to prevent the overreduction of the photosynthetic electron transport chain components. This goal is accomplished by virtue of AOX induction, as well as by export of malate from chloroplasts to mitochondria via the malate-oxaloacetate shunting pathway and through the export of glycolate into peroxisomes, where glycolate is converted to glycine with the subsequent export of glycine to mitochondria, where it is oxidized to serine [50]. The inhibition of AOX in illuminated chloroplasts is accompanied by fast accumulation of NADPH, and consequently, by overreduction of the acceptor side of photosystem I. During germination of *A. thaliana* seeds under continuous strong light, higher rates of ROS production and less efficient use of reducing equivalents in chloroplasts were characteristic of mutants defective in the *Aox1a* gene as compared to wild-type seeds [48, 86]. Thus, AOX along with the aforementioned mechanisms protects plants against light stress and prevents the destruction of the photosynthetic apparatus.

Apart from the aforementioned roles of AOX in optimizing respiratory metabolism, protection against excess ROS, and occasional cell survival, AOX performs other functions according to the stage of development or the metabolic and physiological status of cells, organs, and tissues.

In pathogenic fungi [87] and protists [17], AOX is involved in protection under stresses and ensures the survival of the pathogens during their existence inside the host. In the pathogenic fungus *Sclerotinia sclerotiorum*, AOX participates in regulation of growth, development, and responses to oxidative stress [76].

In the pathogenic yeast *Cryptococcus neoformans*, AOX is expressed in response to changes in temperature of the host organism. A mutant of *C. neoformans* lacking the AOX gene featured low virulence and low tolerance of oxidative stress [72]. Similar data were obtained for the pathogenic fungus *P. brasiliensis* [88], the causative agent of the paracoccidioidomycosis in humans. One of the most important stages in the development of *P. brasiliensis* is the transition of the fungus from the mycelial into the yeast form. This transformation was decelerated after inhibition of AOX or respiratory chain complexes III and IV, and it was completely stopped upon the inhibition of both branches of the respiratory chain, which suggests the possible involvement of AOX in metabolic rearrangements of this pathogenic organism [28].

AOX can participate in antiviral defense. This was shown for tomato and petunia plants [89].

In embryogenesis of carrot (*Daucus carota* L.), the AOX genes *DcAOX1a* and *DcAOX2a* are expressed differentially. During somatic embryogenesis, the gene *DcAOX1a* encoding one AOX isoform is not expressed, whereas the gene *DcAOX2a* encoding the other AOX isoform is expressed very actively. At later stages of embryogenesis, expression of *DcAOX2a* is lowered. The addition of salicylhydroxamic acid, an AOX inhibitor, at the stage of somatic embryogenesis, impaired the development of embryonic structures and retarded the growth of the embryos. This process was reversible and depended on the concentration of the inhibitor added. These results imply the participation of AOX in metabolic rearrangements in plants during embryogenesis and cell differentiation [90].

Thus, AOX increases metabolic plasticity of cells, which could be useful for rapid adaptation to variable nutrient sources or to biotic and abiotic stress factors [5, 91-93]. Different AOX functions might complement each other, rather than be mutually exclusive [6, 79]. Moreover, AOX is presently considered as a marker of stress conditions and as a candidate player involved in cell reprogramming under these conditions [5, 91, 92].

AOX has recently attracted attention of researchers as a possible therapeutic agent for treating disorders in the mitochondrial oxidative phosphorylation system. The genes encoding AOX in the ascidian *Ciona intestinalis* and some Araceae were functionally expressed in cultures of

human cells [94-97]. The expression was found to eliminate lactate accumulation and excessive ROS formation [94, 95], two commonly manifested symptoms of damage to the oxidative phosphorylation system. The AOX expression also prevented the inhibition of growth and removed the elevated sensitivity to prooxidants in cell lines deficient in cytochrome oxidase [96]. Coexpression of NADH-dehydrogenase (Ndi1) from *S. cerevisiae* and AOX from *Emericella nidulans* fully restored the NADH DH/CoQ-reductase and CoQ-oxidase activities in non-viable mice lacking mitochondrial DNA [98]. Expression of AOX of *C. intestinalis* in *D. melanogaster* prevented largely or completely the mortality caused by toxins and by global or partial tissue-specific knockout of COX CoVb and cIV subunits of cytochrome oxidase in the respiratory chain [18], as well as mortality caused by deficiency of Surf1 factor responsible for assembling cytochrome oxidase [99]. The expression also prevented the locomotive defect and excess ROS production in drosophila flies with mutated gene *dj-1β*, the homolog of human gene *DJI* involved in Parkinson's disease [99], and promoted restoration of dopamine-dependent neuroregulation [100]. Expression of AOX in mice had a positive influence on cyanide-resistant respiration in intact organs and facilitated long-term protection against lethal concentrations of cyanide in animals. Furthermore, the enzyme properties, activity of the main respiratory chain components, and the efficiency of the oxidative phosphorylation system in isolated mitochondria remained unchanged [101].

These data indicate that AOX might be an important tool to overcome the adverse aftereffects of restricted activity of the main respiratory chain in cells and animals. The presence of active AOX provides not only the bypass of a defective cytochrome pathway, but also ensures the sustained activity of the tricarboxylic acid cycle under constrained activity of the main cytochrome chain [102] and alleviates cell damage caused by mitochondrial ROS. It should be remembered that mitochondrial dysfunctions and overproduction of superoxide in mitochondria is an important factor in many human diseases, ranging from systemic pathology in children to cardiomyopathy, ischemia, cancer, and neurodegenerative disorders.

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