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Classical and alternative components of the mitochondrial respiratory chain in pathogenic fungi as potential therapeutic targets

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Abstract The frequency of opportunistic fungal infection has increased drastically, mainly in patients who are immunocompromised due to organ transplant, leukemia or HIV infection. In spite of this, only a few classes of drugs with a limited array of targets, are available for antifungal therapy. Therefore, more specific and less toxic drugs with new molecular targets is desirable for the treatment of fungal infections. In this context, searching for differences between mitochondrial mammalian hosts and fungi in the classical and alternative components of the mitochondrial respiratory chain may provide new potential therapeutic targets for this purpose.

Keywords Fungal Mitochondria · Fungal infection · Therapeutic targets · Antifungal

Introduction

Fungi represent a group of eukaryotic organisms that are well adapted to a diversity of environments. Fungi are important for the recycling of carbon and nitrogen, food, enzymes, and biodetergents, as well as for antibiotic production. On the other hand, fungi can be dangerous contaminants of grains and seeds because of their potential for mycotoxin production, and fungi are responsible for several animal and plant diseases. In the past few decades, the frequency of opportunistic fungal infection has increased drastically, mainly in patients who are immunocompromised due to organ transplant, leukemia or HIV infection (Walsh and Groll 1999; Groll and Walsh 2001; Brakhage and Liebmann 2005). *Candida* spp. and *Aspergillus* spp. remain the most frequent causes of invasive fungal infection (Richardson and Lass-Flörl 2008). Furthermore, *Cryptococcus neoformans* is the most common cause of meningoencephalitis in HIV-infected patients (Groll and Walsh 2001). Other fungi and yeast, such as *Fusarium* spp., *Zygomyces, Trichosporon* and *Coccidioides immitis*, have also emerged as opportunistic fungi in immunocompromised patients (Shao et al. 2007).

In spite of the increase in fungal infections, only a few classes of drugs (with a limited array of targets) are available for antifungal therapy; these drugs include polyenes, azoles, allylamines, nucleoside analogs and the recently introduced echinocandins. For many years, amphotericin B was considered the "gold standard" treatment for invasive fungal infection (Petrikkos and Skiada 2007). However, this drug presents undesirable side effects including fever, nausea, vomiting and nephrotoxicity (Bates et al. 2001). Therefore, many of these classes of drugs have limited use, making the discovery of new, more specific and less toxic drugs with new molecular targets desirable for the treatment of invasive fungal infections. Along those lines, mitochondria are potential targets, because they are present in most eukaryotic cells. In addition to being responsible for more than 90% of cellular ATP production, these organelles play several roles, including generation and regulation of reactive oxygen species, calcium homeostasis, programmed cell death and metabolic processes (involving

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amino acids, lipids, nucleotides and iron) (Newmeyer and Ferguson-Miller 2003; Pagliarini and Dixon 2006).

Classical components of the mitochondrial respiratory chain (MRC)

ATP production is coupled to the electron transport chain in the inner mitochondrial membrane. The classical mitochondrial respiratory chain is composed of Complexes I and II, Ubiquinone or Coenzyme Q, Complex III, Cytochrome *c*, and Complex IV, which provides the terminal reduction of oxygen into water (Rosenfeld and Beauvoit 2003). The energy released from electron transport between NADH and $FADH_2$ and molecular oxygen in each complex is used to pump protons across the inner mitochondrial membrane, creating an electrochemical gradient ($\Delta = \Delta\psi + \Delta\mu^{H+}$; Wallace 2010). According to the chemiosmotic model (Mitchell 1961), the electrochemical proton gradient drives ATP synthesis via the flow of protons through ATP synthase (Complex V).

MRC Complex I

NADH, produced by the breakdown of carbohydrates, fats and proteins, is oxidized by mitochondrial NADH:ubiquinone oxidoreductase (Complex I). Two electrons from NADH oxidation are able to reduce ubiquinone to ubiquinol. This electron transfer is coupled to translocation of four protons across the inner mitochondrial membrane (Wikström 1984; Brown and Brand 1988) and contributes to the proton electrochemical gradient (Videira 1998). Complex I can be inhibited by more than 60 different families of compounds (Lenaz and Genova 2010), such as rotenone, piericidin A and B, ibedenone, amythal, barbiturates, Demerol, mercurials and insecticides (Hatefi 1985).

Mitochondrial NADH:ubiquinone oxidoreductase is one of the largest and most complicated complexes of the inner membrane-bound complexes, with an L-shaped structure (Grigorieff 1999; Hirst et al. 2003). The subunit content in this complex can vary among different species (Gabaldon et al. 2005). There are 14 subunits in bacteria (Weidner et al. 1993; Xu et al. 1993), 30 in algae (Cardol et al. 2004), 35 to 37 in fungi (Weiss et al. 1991; Videira 1998; Abdrakhmanova et al. 2004), 40 in plants (Rasmusson et al. 2008) and 46 in mammals (Gabaldon et al. 2005). In spite of the high similarity between the complexes found in fungi and higher eukaryotes, at least 3 proteins (out of 28) appear to be fungus-specific (Videira and Duarte 2001; Gabaldon et al. 2005). The majority of multiprotein subunits of Complex I are encoded by the nucleus, synthesized in the cytoplasm and imported into mitochondria (Videira and Duarte 2001). In fungi, similar to other organisms, at least seven subunits are encoded by mitochondrial DNA (Videira and Werner 1989; Woo et al. 2003; Tambor et al. 2006; Cardoso et al. 2007).

MRC Complex I and reactive oxygen species generation

Mitochondria are an important source of reactive oxygen species (ROS) in the majority of cell types (Cadenas and Davies 2000; Turrens 2003; Kowaltowski et al. 2009). In the respiratory chain, superoxide is primarily generated by Complexes I and III (Lambert and Brand 2004; Skulachev 2006) and is converted to hydrogen peroxide by superoxide dismutase (SOD, Forman and Kennedy 1974; Loschen et al. 1974). Hydrogen peroxide is able to generate hydroxyl radicals by the Fenton/Haber-Weiss reaction. ROS may cause significant damage to cellular proteins, lipids, and DNA. In addition, ROS generated by mitochondria are able to act as signaling molecules in the cytosol, regulating cell cycle, stress response, energy metabolism and redox balance (Kowaltowski et al. 2009). Interestingly, most pathogenic fungi are able to adapt to oxidative stress by increasing their antioxidant defense systems. Drugs specifically inhibiting Complex I can overload ROS production in mitochondria; several reports indicate that chronic oxidative stress may delay systemic invasion of the host (Fekete et al. 2007; Fekete et al. 2008). In this sense, Complex I has emerged as a possible target for both insecticides (Friedrich and Böttcher 2004) and treatment for fungal infection.

MRC Complex II

Succinate dehydrogenase, a component of Complex II in the mitochondrial respiratory chain and a membrane-bound component involved in the tricarboxylic acid cycle (Cecchini 2003), catalyzes the oxidation of succinate to fumarate. After succinate oxidation, succinate dehydrogenase transfers electrons to the ubiquinone pool (Saliola et al. 2004). Complex II has been found in the mitochondria of mammals, parasites, protozoans (Vercesi et al. 1998; Uyemura et al. 2000; Kita et al. 2002), yeast and filamentous fungi (Tudella et al. 2004; Cavalheiro et al. 2004; Lemire and Oyedotun 2002; Martins et al. 2008). Succinate dehydrogenase is highly conserved and consists of four subunits, two catalytic and two structural, all encoded by the nuclear genome (Oyedotun and Lemire 1999; Saliola et al. 2004; Rutter et al. 2010). In contrast to other mitochondrial complexes, eukaryotic Complex II is the only mitochondrial complex unable to pump protons across the inner mitochondrial membrane.

Succinate dehydrogenase can be regulated by post-translational phosphorylation and acetylation, as well as by active site inhibition (Rutter et al. 2010). Malonate, oxaloacetate (Ackrell et al. 1974), 3-nitropropionate (Alston et al. 1977), thenoyl trifluoroacetone and carboxin (Mowery et al. 1977; Matsson and Hederstedt 2001) can serve as inhibitors of succinate dehydrogenase. Recently, it has been demonstrated that siccanin, a compound isolated from *Helminthosporium siccans* Drechsler culture broth, exhibits potent antifungal activity, particularly against several pathogenic fungi such as *Trichophyton, Epidermophyton* and *Microsporum* (Mogi et al. 2009).

MRC Complex III

Ubiquinol:cytochrome *c* oxidoreductase (Complex III or *bc*1 complex) transfers electrons from ubiquinol to ferricytochrome *c* (Hunte et al. 2003; Crofts 2004). This electron transfer is coupled to the vectorial translocation of protons across the inner mitochondrial membrane, contributing to generation of the electrochemical proton gradient (Zara et al. 2009). Mitochondrial cytochrome *bc*1 has a dimeric, pear-shaped structure (Schultz et al. 2001) containing 10 protein subunits in yeast (Zara et al. 2009) and *Plasmodium* (Barton et al. 2010) or 11 subunits in mammalian heart and liver (Vázquez-Acevedo et al. 1993). Major subunits are nuclear-encoded; only the cytochrome *b* subunit is encoded by mitochondrial DNA in all eukaryotes (Yu et al. 1998).

In yeast, the catalytic core of Complex III is composed of three subunits: cytochrome *b*, cytochrome *c1* and the Rieske iron-sulfur protein, which are highly conserved across different species (Rieske 1976; Degli-Esposti et al. 1993). The mechanism by which the *bc*1 complex carries out this energy-transducing electron transfer from ubiquinol to cytochrome *c* is known as the protonmotive Q cycle (Mitchell 1976). The cytochrome *bc*1 complex has two quinone binding sites: the Qo site, where ubiquinol is oxidized, and the Qi site, where ubiquinone is reduced (see Hunte et al. 2003; Zhang et al. 2008). The *bc*1 Complex is inhibited by antimycin A, myxothiazol and stigmatellin, which abolish the proton motive activity of Complex III and block the transfer of electrons to the downstream components of the respiratory chain (Slater 1973; Lemesle-Meunier 1989; Gurung et al. 2008; Barton et al. 2010). Complex III is a well-established source of mitochondrial ROS (Turrens et al. 1985; Dröse and Brandt 2008; Kowaltowski et al. 2009). Antimycin A inhibits electron transfer from Qp to the Qn site of cytochrome *b*, which causes the accumulation of semiquinone intermediates, increasing ROS generation. Myxothiazol and stigmatellin bind the domain proximal to heme *b*L but do not interact with the Rieske iron-sulfur protein, preventing the formation of semiquinone intermediates and subsequent ROS generation. However, this binding may increase release of ROS from Complex I (Kowaltowski et al. 2009).

Based on their mechanisms of action, drugs targeting Complex III could be efficient tools for pathogen control. Several compounds have been used as inhibitors of Complex III in certain microorganisms, including the following combinations: (*i*) atovaquone against *Toxoplasma gondii* (Baggish and Hill 2002) and *Pneumocystis* sp. (Walker and Meshnick 1998; Kessl et al. 2004); (*ii*) 2-hydroxy-naphthoquinone, pyridones, quinolones, acridones, acridine diones and rhinacanthin against malaria parasites (Vaidya and Mather 2000; Kessl et al. 2007; Barton et al. 2010; Kongkathip et al. 2010); (*iii*) azoxystrobin, dimoxystrobin, enestroburin, famoxadone, fenamidone, fluoxastrobin, kresoxim-methyl, metominostrobin, orysastrobin, picoxystrobin, pyraclostrobin, pyribencarb and trifloxystrobin against fungal plant pathogens (Fisher and Meunier 2008); (*iv*) diuron and illicicolin H against *Saccharomyces cerevisiae* (Di Rago and Colson 1988; Gutierrez-Cirlos et al. 2004) and *Candida parapsilosis* (Camougrand et al. 1986) and (*v*) ascochlorin against *Pichia (Hansenula) anomala* (Berry et al. 2010). However, the structure of cytochrome *bc*1 is highly conserved among species, so these drugs can also be toxic to mammalian hosts. On the other hand, there are some sequence differences that may be useful when searching for specific inhibitors with differential reactivity toward pathogens and mammals (Fisher and Meunier 2008).

MRC Complex IV

Cytochrome *c* oxidase (COX) (Complex IV) catalyzes the last step of mitochondrial respiration, the reduction of molecular oxygen to water, concomitant with the oxidation of cytochrome *c*. As with Complex I and Complex III, the transfer of electrons is coupled to proton translocation from the matrix into the intermembrane space. This transfer contributes to the electrochemical gradient, which can be used for ATP synthesis by ATP synthase (Complex V).

COX is a multimeric enzyme and belongs to the terminal heme-copper oxidase superfamily, which contains 13 subunits in mammals (Burke and Poyton 1998), 11 in *S. cerevisiae* (Gier et al. 1995), 7 in *Dictyostelium discoideum* (Bisson et al. 1997; Ludwig et al. 2001) and 4 in *Paracoccus denitrificans* (Iwata et al. 1995). In mammals and yeast, three subunits are encoded by the mitochondrial genome, whereas the remaining subunits are encoded by the nuclear genome, translated in the cytoplasm and imported into mitochondria (Bandeira and Nobrega 2008). The three subunits encoded by the mitochondrial genome form the catalytic core of the

complex and contain metal prosthetic groups. Copper and heme A form three redox centers: a CuA center in subunit 2 and a heme *a* and a CuB-heme *a3* binuclear center in subunit 1 (Fontanesi et al. 2008).

COX is inhibited by cyanide, sulfide, carbon monoxide and nitric oxide (Cooper and Brown 2008). Currently, there are no COX inhibitors being used for antifungal therapy. However, cytochrome *c* oxidase is considered a key component of the regulation of oxidative phosphorylation, and COX deficiency has been associated with several diseases, such as Leigh syndrome (Tiranti et al. 1998; Zhu et al. 1998), fatal and benign infantile myopathies (Burke and Poyton 1998), Alzheimer's and Parkinson's diseases (Wallace 1992; Diaz 2010) and Huntington's disease (Zuccato et al. 2010). Because of the sequence differences in cytochrome *c* oxidase subunits in mammals and fungi, these proteins represent a potential target for the treatment of fungal infections.

Alternative components of the MRC

In addition to the classical components of the mitochondrial respiratory chain, plants, fungi and some protozoans possess alternative components, such as an alternative NADH dehydrogenase, an alternative oxidase and uncoupling protein (UCP).

Alternative NADH dehydrogenase

Three groups of NADH:ubiquinone oxidoreductases are known: the proton-translocating NADH:ubiquinone oxidoreductase (designated Complex I in mitochondria and NDH-1 in bacteria), the Na^+-translocating NADH:ubiquinone oxidoreductase (only documented in bacteria), and NDH2, an alternative NADH:ubiquinone oxidoreductase. NDH2 catalyzes the same redox reaction as the respiratory chain Complex I but does not contribute to the generation of a transmembrane proton gradient (Kersher 2000); it is rotenone-insensitive and flavone-sensitive. In contrast to Complex I, which contains at least 35 subunits (Joseph-Horne et al. 2001), NDH2 is a single polypeptide. The respiratory chain of *S. cerevisiae* lacks Complex I, instead containing three rotenone-insensitive NADH:ubiquinone oxidoreductases: one internal rotenone-insensitive NADH: ubiquinone reductase (Ndi1) (Kerscher 2000) facing the matrix and two external rotenone-insensitive NADH:ubiquinone oxidoreductases (Nde1 and Nde2) facing the intermembrane space. In addition to the two external Nde proteins, *Neurospora crassa* possess a third external Nde localized both to the mitochondria and the cytoplasm (Carneiro et al. 2007).

Alternative dehydrogenases have been described in several fungi, such as *S. cerevisiae* (Small and Mcalister-Henn 1998), *Yarrowia lipolytica* (Kerscher et al. 1999), *Kluyveromyces lactis* (Tarrio et al. 2006) and *Aspergillus fumigatus* (Tudella et al. 2004). Although the precise physiological role of NDH2 is still unknown, it appears to be related to the regulation of intracellular redox balance, energy production (Tarrio et al. 2006) and cold stress (Svensson et al. 2002).

Because NDH2 is absent in mammalian hosts, it is an attractive target for new antifungal agents (Lin et al. 2008). Alternative NADH dehydrogenases are present in most fungi and protozoans. Inhibitors of NDH2 have been primarily studied in parasites; these inhibitors include (*i*) 2-heptyl-4-hydroxyquinoline N-oxide (HQNO), 5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin), diphenyleneiodonium chloride and diphenyliodonium chloride for *Plasmodium falciparum* (Fry et al. 1990, Biagini et al. 2006); (*ii*) 1-hydroxy-2-dodecyl-4(1)quinolone (HDQ) for *T. gondii* (Lin et al. 2008) and *S. cerevisiae* (Yamashita et al. 2007) and (*iii*) scopafungin, HQNO and aurachin C in *Mycobacterium smegmatis* and *Plasmodium yoelii* (Mogi et al. 2009).

Alternative ubiquinol oxidases

Cyanide-resistant alternative oxidases (AOXs) are terminal ubiquinol oxidases that directly transfer electrons from ubiquinol to oxygen, reducing oxygen to water. AOXs bypass electron transfer through mitochondrial Complexes III and IV, lowering the proton motive force across the inner mitochondrial membrane and affecting ATP production (Moore and Siedow 1991). An important characteristic of AOX activity is the capacity to maintain oxygen consumption in spite of inhibition of Complex III by antimycin A or inhibition of Complex IV by cyanide (Beconi et al. 1983; Bendall and Bonner 1971). Alternatively, AOX activity can be blocked by salicylhydroxamic acid, benzohydroxamic acid and *n*-propyl gallate (Lambowitz and Slayman 1971; Vianello et al. 1997; Jarmuszkiewicz et al. 1998; Siedow and Girvin 1980).

AOXs are members of the di-iron protein family, which includes methane monooxygenase, the R2 subunit of ribonucleotide reductase, stearoyl-acylcarrier desaturase (Δ9-desaturase), rubrerythrin, hemerythrin and ferritins (Albury et al. 2009). For these proteins, the active site is covered in a four-helix structure containing two conserved EXXH motifs, with the glutamate and histidine residues coordinating iron atoms (Siedow et al. 1995; Moore et al. 1995; Albury et al. 2009). The quinone and oxygen binding sites are also thought to be located in close proximity to the four-helix bundle (Affourtit et al. 2002; Berthold et al.

2000; Umbach et al. 2002; Berthold and Stenmark 2003; Stenmark and Nordlund 2003: Abramson et al. 2000; Moore and Albury 2008). To date, the lack of crystallographic structures for AOXs makes it difficult to establish their catalytic mechanism.

AOXs have been documented in all higher plants studied so far (Albury et al. 2009), as well as in other organisms including algae (Tischner et al. 2004), yeast (Veiga et al. 2003), free-living amoebae (Jarmuszkiewicz et al. 2002), protozoa (Suzuki et al. 2005), proteobacteria (Atteia et al. 2004; Stenmark and Nordlund 2003), animal species (McDonald et al. 2009; McDonald and Vanlerberghe 2006) and microsporidia (Williams et al. 2010). The importance of AOXs is related to their presence in human fungal pathogens such as *Candida albicans* (Huh and Kang 1999; Huh and Kang 2001), *A. fumigatus* (Tudella et al. 2004), *Histoplasma capsulatum* (Johnson et al. 2003) and *Paracoccidioides brasiliensis* (Medoff et al. 1987; Martins et al. 2008).

Unlike plant AOXs, which function as homodimers, fungal AOXs are present as monomers and lack the conserved cysteine residues observed in the plant enzymes (Umbach and Siedow 2000). Fungal AOXs also differ from their plant counterparts by containing an insertion of 20 to 25 amino acids before helix I and by being regulated by purine nucleotides instead of α-keto acids (known inducers of plant AOXs) (Umbach and Siedow 2000). Functionally compromised mitochondria (Chae and Nargang 2009) and ROS can act as signals to increase AOX expression in many fungal species, such as *Magnaporthe grisea* (Yukioka et al. 1998), *N. crassa* (Tanton et al. 2003), *A. fumigatus* (Magnani et al. 2007), *C. albicans* (Hwang et al. 2003), *H. capsulatum* (Johnson et al. 2003) and *P. brasiliensis* (Martins et al. 2010). Consequently, AOXs defend the cell against oxidative stress, as indicated by the low levels of reduced ubiquinone maintained by AOX activity (Maxwell et al. 1999; Moller 2001; Rhoads and Subbaiah 2007).

Inhibition of Complex III and/or AOX, which interferes with ROS homeostasis, was observed to affect the mycelium-to-yeast transition in *P. brasiliensis* (Martins et al. 2010). The inhibition of AOX has been reported to affect the development and/or differentiation of several pathogens, such as *C. albicans* (Ruy et al. 2006), *C. parvum*, *T. gondii* (Roberts et al. 2004), and *Trypanosoma brucei brucei* (Nihei et al. 2003); however, it is still unclear whether the killing of these microorganisms through mitochondrial respiratory chain inhibition occurs due to energy deficiency, ROS generation and cell damage, or both.

AOX enzymes may have a role in immune evasion by pathogens because they confer resistance against nitric oxide (NO), a potent inhibitor of the cytochrome pathway produced by cells of the mammalian immune system (Missall et al. 2004). It has been proposed that the insensitivity of AOXs to NO is due to a change in the position of a tyrosine residue from helix II to helix III (Moore and Albury 2008). We previously demonstrated down-regulation of the *Af*aox gene by RNA silencing techniques in *A. fumigatus*. The decrease in AOX gene expression caused an increase in ROS production and a higher susceptibility to oxidative stress challenges *in vitro.* In addition, this strain was less protected against macrophage killing (Magnani et al. 2009), suggesting that AOX is required for *A. fumigatus* survival during host infection and for resistance to ROS generated by macrophages. The important roles that AOXs play in the defense of pathogens against cellular damage and their absence from the mammalian hosts make them attractive targets for antimicrobial therapy. Indeed, ascofuranone, a potent inhibitor of AOX in *T. brucei* (Minagawa et al. 1996; Yabu et al. 1998) is in clinical trials (Yabu et al. 2003).

Uncoupling proteins

Uncoupling mitochondrial proteins (UCPs) belong to the anion carrier protein family located in the inner mitochondrial membrane. UCPs can dissipate the electrochemical proton gradient generated by respiratory chain (Nicholls and Rial 1999) bypassing the ATP synthase. UCP1 was first described in brown adipose tissue (Ricquier and Kader 1976) and four other UCP homologous have been identified in mammalians (Ricquier and Bouillaud 2000). Vercesi et al. (1995) described an UCP out of mammals, in potato tuber, and after that UCPs have been described in other organisms such as insects (Fridell et al. 2004), protozoa (Jarmuszkiewicz et al. 1999; Uyemura et al. 2000; Jarmuszkiewicz et al. 2002) and fungi (Jarmuszkiewicz et al. 2000; Cavalheiro et al. 2004; Tudella et al. 2004; Luévano-Martínez et al. 2010).

Several genes encoding proteins sharing a significant degree of identity with UCP1 have been identified. UCPs and plant UCPs (PUMP) contain 295–300 amino acid residues and exhibit a tripartite structure consisting of three repeated domains of 100 residues, with two hydrophobic transmembrane alpha-helice regions linked by a hydrophilic loop orientated to matrix side (Vercesi et al. 2006). Each domain contains specific sequence motifs of the Energy Transfer Protein Signature, identified both in animal and plant UCPs (Borecký et al. 2001). UCP1-UCP3 and PUMP have molecular masses of 31-34 kDa; UCP4 and UCP5 are larger proteins with masses of 36–38 kDa (Vercesi et al. 1995; Ledesma et al. 2002).

UCPs are encoded by the nuclear genome and do not have an amino-terminal cleavable import sequence to drive their import into mitochondria (Ledesma et al. 2002). The

import depends on the amino-terminal domain, which contains targeting information to the outer membrane and interacts with the receptor protein hTom20; it exhibits binding characteristics of an internal targeting signal. The second transmembrane domain and in the central matrix loop, is the critical one for targeting and insertion into the inner membrane (Schleiff and McBride 2000).

UCPS in mammals, plant and fungi are regulated by free fatty acids and inhibited by purine nucleotides (Klingenberg 1988; Klingenberg 1990; Skulachev 1991; Echtay 2007). However, little is known about the physiological regulation of UCP in unicellular eukaryotes. It is believed it includes a protection against ROS production (Jarmuszkiewicz et al. 2010). In the amoeboid *Acanthamoeba castellanii*, for example, UCP inhibition by GDP increases ROS and UCP activation by free fatty acid decreases ROS (Czarna and Jarmuszkiewicz 2005). In fungi, mitochondrial uncoupling by UCP and AOX increases the resistance killing by phagocytose during host invasion (Cheng et al. 2007). Therefore, although UCPs represent a potential target for the treatment of fungal infections, to date there is no natural or synthetic compound being used for this purpose.

Conclusions

In recent years, considerable advances have been made in the development of new drugs such as echinocandins for the treatment of invasive fungal infection. However, these emerging fungal pathogens are also more resistant to standard antifungal agents. Thus, the challenge remains to discover new drugs for the treatment of fungal infections as well as different targets for new drugs, raising the possibility of antifungal drug combinations. Along those lines, searching for differences between mitochondrial mammalian hosts and fungi in the classical and alternative components of the mitochondrial respiratory chain may provide new potential therapeutic targets in treating pathogenic fungal infections.

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