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# Functions of fungal melanin beyond virulence

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## Abstract

Melanins are ancient biological pigments found in all kingdoms of life. In fungi, their role in microbial pathogenesis is well established; however, these complex biomolecules also confer upon fungal microorganisms the faculty to tolerate extreme environments such as the Earth's poles, the International Space Station and places contaminated by toxic metals and ionizing radiation. A remarkable property of melanin is its capacity to interact with a wide range of electromagnetic radiation frequencies, functioning as a protecting and energy harvesting pigment. Other roles of fungal melanin include scavenging of free radical, thermo-tolerance, metal ion sequestration, cell development, and mechanical-chemical cellular strength. In this review, we explore the various functions ascribed to this biological pigment in fungi and its remarkable physicochemical properties.

## Graphical Abstract

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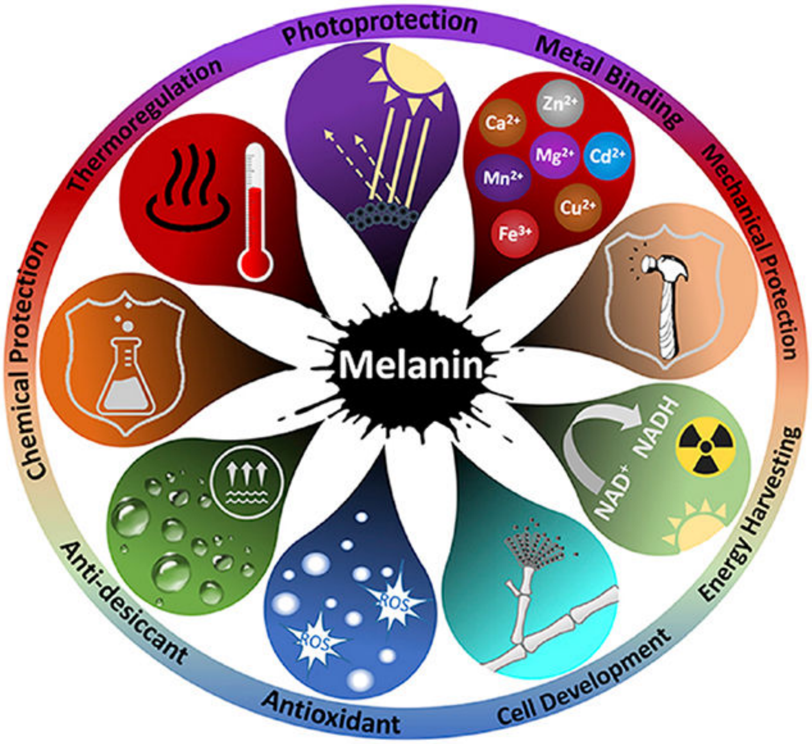
Declaration of interest
The authors declare no competing interests.



## Abstract

Functions of fungal melanin.

Fungal melanins play multiple biological functions including photoprotection, energy harvest and thermoregulation by readily absorbing and transducing electromagnetic radiation. Fungal melanins also function in free radical and metal binding; protection against dehydration, chemical and mechanical stressors; and fungal development and conidiation.

### Keywords

fungal melanization; thermal melanism; energy transducer; fungal melanin; fungal pigments; black fungi; energy harvest; free radical scavenging

## Introduction

Pigments are produced by most living organisms and give our world a variety of colors through the absorption and refraction of specific wavelengths of light. There are many kinds of biological pigments in nature, ranging from monomeric (i.e. carotenoids, luciferin, flavonoids, and heme/porphyrin-based, such as chlorophylls, bilirubin, hemoglobin, hemocyanin) to polymeric (i.e. melanins, tannins, and humic substances). All pigments contain conjugated moieties (i.e. aromatics rings) that allow electronic resonances and mediate energy transfer reactions in cells. The capture and/or reflected radiation energy by pigments serves multiple biological functions ranging from camouflage or makeup to fundamental roles in the maintenance of life including harnessing solar energy for metabolic use and protection against radiation damage.

Among the biological pigments, melanins represent a unique class. Historically, melanins have been difficult to define and categorize due to their diversity and structural complexity. Melanins can be classified into eumelanins, pheomelanins, neuromelanin and allomellalins (Ambrico, 2016). All are heterogeneous polyphenols that form higher-order structures with unique physicochemical properties, including broadband optical absorption, paramagnetism, charge transport and remarkable structural stability. These properties allow melanins to perform diverse functions in biological systems and melanization represents a general adaptation mechanism to climate changes (Roulin, 2014). The widespread presence of melanins in biology implies a functional importance for this class of biomolecules in the evolution of life on Earth.

In the fungal kingdom, melanization is observed across all phyla. Some fungal species are constitutively melanized while others melanize only under specific developmental phases (i.e. conidia, yeast filamentous growth), in response to environmental queues, and/or in the presence of phenolic melanin precursors (Bell and Wheeler, 1986). Fungal species that are constitutively melanized are referred to as melanotic, black, dematiaceous, microcolonial or meristematic fungi. Those fungal species that only melanized under certain conditions could be termed “facultative melanotic” fungi. Melanotic fungi appear to be phylogenetically diverse (Sterflinger et al., 1999) with a worldwide distribution, typically colonizing harsh environmental niches not suitable to most life forms (Table 1). Such environments are characterized by diverse conditions of drastic temperature fluctuations, elevated radiation exposure, high osmotic pressure, oxidative stresses, low water activity and nutrient availability. Melanization allows these microorganisms to tolerate the various physical and chemical stresses from their surroundings (Dadachova and Casadevall, 2008; Gessler et al., 2014; Robinson, 2001) making them polyextremophiles.

Most fungal melanins are generated from the polymerization of 1,8-dihydroxynaphthalene (DHN), but fungal species can also utilize other pigment precursors such as: tyrosine, gamma-glutaminyl-4-hydroxybenzene (GHB), catechol, homogentisic acid, catecholamines, and (+)-scytalone (Bell et al., 1976; Bell and Wheeler, 1986; Belozerskaya et al., 2016; Solano, 2014; Weijn et al., 2013; Wheeler and Bell, 1988). During melanin synthesis, the phenolic precursors undergo multiple oxidation and reduction steps, which can occur enzymatically or passively by spontaneous polymerizations. In fact, a translucent L-Dopa aqueous solution will eventually precipitate into melanin particles at room temperatures even in the absence of enzymes (Mason, 1955; Nosanchuk et al., 2001; Soares Bronze-Uhle et al., 2015). Melanin biosynthesis involves multiple enzymes including polyphenoloxidases (i.e. tyrosinase, laccase, catechol oxidase)- key enzymes that carry out the rate-limiting initial oxidations of phenolic melanin precursors (Bell et al., 1976; Bell and Wheeler, 1986; d’Ischia et al., 2013; Eisenman and Casadevall, 2012; Ito and Wakamatsu, 2008; Solano, 2014; Wheeler and Bell, 1988). Their activity depends on copper ions at their catalytic site, which help orient the reducing substrate and coordinate molecular oxygen for catalysis (Cowley et al., 2016; Decker and Tuczek, 2000; Gasparetti and tutkimuskeskus, 2012; Goldfeder et al., 2014). Thus, copper homeostasis is key for fungal melanin biosynthesis

(Mauch et al., 2013; Upadhyay et al., 2013). The biosynthesis of fungal melanins was previously reviewed and will not be discussed in depth here (Bell and Wheeler, 1986; Bultler, 1987; Eisenman and Casadevall, 2012; Henson et al., 1999; Langfelder et al., 2003; Ma and Sun, 2012; Nosanchuk et al., 2015). It has been suggested that fungal melanins, regardless of their precursor, may share similar functional groups and comparable physicochemical properties (Fogarty and Tobin, 1996). However, such properties have yet to be studied in the various fungal melanins. Given the large number of melanotic species and biosynthetic pathways for melanin production, it is likely that fungal melanins express a variety of structural and chemical characteristics that remain to be defined.

The structure of fungal melanin, or melanin in general, remains poorly understood (Nosanchuk et al., 2015; Solano, 2014, 2016). Like other amorphous substances in nature (i.e. wood), an unambiguous determination of its higher-order structural conformations (analogous to secondary, tertiary, and/or quaternary structures) is beyond our current technological and analytical horizons. Our present understanding of the structure and properties of melanin originate primarily from studies on synthetic melanins and/or non-fungal natural melanins (mainly from squid/cuttlefish), which are not identical, but do share physicochemical characteristics (Abbas et al., 2009; Ambrico, 2016; d'Ischia et al., 2009; Duff et al., 1988; Haywood et al., 2006; Ligonzo et al., 2009; Meredith and Sarna, 2006). One model of melanin structure suggests that indolic and/or phenolic monomers are polymerized into a series of ordered planar arrangements of regularly-interspaced stacked layers similar to graphite, that can cross-link into more heterogeneous and disordered macromolecular configurations (Kim et al., 2016; Meredith and Sarna, 2006; Nosanchuk et al., 2015). This is known as the local-order-global-disorder model of melanin; it involves a combination of $\pi$-stacking, hydrogen and ionic bonded nanostructures of unclear dimensions, which then aggregate to form disordered particles with spherical dimensions known as melanin granules (Bridelli, 1998; Chen et al., 2014; Clancy et al., 2000; Kim et al., 2016; Meredith and Sarna, 2006; Nosanchuk et al., 2015; Tran et al., 2006; Watt et al., 2009). High-resolution microscopy of fungal melanins also show granular patterns and X-ray diffraction analysis has produced patterns consistent with a stacked planar sheet structure with inter-sheet distances of approximately 0.4 nanometers (Bayry et al., 2014; Casadevall et al., 2012; Franzen et al., 2008b; Franzen et al., 1999; Gomez et al., 2001; Morris-Jones et al., 2005; Morris-Jones et al., 2003; Nosanchuk et al., 2002; Walker et al., 2010). The mean size, mass, and unit of fungal melanin granules remain unknown.

In fungi, melanin may be contained at the cell surface or released into the extracellular space (Dong and Yao, 2012; Doss et al., 2003; Gadd and de Rome, 1988; Hegnauer et al., 1985; Jalmi et al., 2012). The exact location of melanin granules at the cell surface varies between fungal species. For instance, in *Cryptococcus neoformans,* melanin granules are deposited between the plasma membrane and the innermost part of the cell wall (Eisenman et al., 2005). In other fungal species, melanin inserts within or at the surface of the cell wall matrix (Caesar-Tonthat et al., 1995; Carzaniga et al., 2002; Ellis and Griffiths, 1974, 1975; Gadd and Griffiths, 1980; Kogej et al., 2007). Melanin deposition in the fungal cell wall involves intimate molecular interactions with chitin structures (Baker et al., 2007; Banks et al., 2005; Bull, 1970; Chatterjee et al., 2015; Tsirilakis et al., 2012; Walker et al., 2010; Walton et al., 2005; Wang et al., 1999). Disruption of chitin metabolism results in a "leaky melanin" phenotype, where the pigment is no longer contained in the cell wall and is release to the extracellular milieu (Baker et al., 2007; Banks et al., 2005) (Baker et al., 2007; Banks et al., 2005; Tsirilakis et al., 2012). Traces of chitin Nuclear Magnetic Resonance (NMR) signatures are always detected in melanin isolates from *C. neoformans* (Chatterjee et al., 2015), which means these polysaccharides are in close association with the pigment such that they resist the hydrolysis steps during melanin preparations (Wang et al., 1996). Other biomolecules such as lipids, peptides, and carbohydrates are also detected in Cryptococcal melanin purifications, but their identification and relevance in melanogenesis are still unknown (Chatterjee et al., 2015; Tian et al., 2003; Zhong et al., 2008).

The synthesis of fungal melanin is similar to animal melanogenesis in that it occurs inside lipids vesicles or melanosomes (Eisenman et al., 2009; Franzen et al., 2008a; Upadhyay et al., 2016; Walker et al., 2010). This is likely to protect the cell from the highly reactive free radical phenolic intermediates produced during intracellular melanogenesis and vesicular structures may be necessary to contain and provide shape to the products of a free radical reaction. Evidence for fungal melanosomes include: (i) the melanin coat is visibly formed by layers of spherical melanin granules with size dimensions that are comparable to fungal vesicles (Alviano et al., 1991; Eisenman et al., 2005; Hegnauer et al., 1985; Morris-Jones et al., 2005; San-Blas et al., 1996; Walker et al., 2010); (ii) NMR of melanin isolates contain lipid signatures (Alviano et al., 1991; Chatterjee et al., 2015; Chatterjee et al., 2014; Eisenman et al., 2009); (iii) laccase, the enzyme that catalyzes melanin formation, is found within vesicles (Rodrigues et al., 2008), and (iv) isolated vesicles can melanized in the presence of L-Dopa (Eisenman et al., 2009). A recent study showed that melanin synthesis in *Aspergillus* starts within intracellular endosomes that are secreted unconventionally into the cell wall, where additional melanin biosynthetic enzymes can accumulate (Upadhyay et al., 2016). Laccase localizes at the cell wall in a pH-dependent manner (Panepinto and Williams, 2007; Zhu et al., 2001) consistent with the possibility of *in situ* melanogenesis at the fungal cell wall.

## Functions of melanin in fungal biology

Melanins exhibit physicochemical and structural characteristics not replicated by any other pigment or biomolecule. The inherent complex nature of melanin limits our capacity to elucidate their higher-order structure and therefore understand their functions. Over recent years, physicochemical studies on synthetic and non-fungal natural melanins have provided valuable insights about the properties underlying their multiple biological functions in eukaryotic systems (Abbas et al., 2009; d'Ischia et al., 2009; Meredith and Sarna, 2006). Elucidation of the physiochemical properties of fungal melanins is necessary requirement to better understand the diverse functions of melanin in fungal biology.

The wide geographical distribution of black fungi suggests that melanins provide these organisms with special properties that translate into survival and/or adaptation advantages in extreme environmental conditions. The contribution of melanins to fungal virulence and human pathogenesis has stimulated significant functional characterization studies. Fungal melanin is considered an important virulence factor in a number of fungal species, acting as non-specific armor during infection that protects the fungus against host immune mechanisms (Butler et al., 2001; Nosanchuk and Casadevall, 2003; Perfect et al., 1998). However, fungal melanization clearly serves many biological purposes outside the human host, including photoprotection, energy harvest, free radical quenching, protection against heat and cold stress, metal chelation, cell strength, resistance to desiccation and cell development.

In the following sections, we discuss the various functions of fungal melanins in light of some general physicochemical properties described for melanins of non-fungal origin.

## Photoprotection

Electromagnetic radiation is both necessary (e.g. photosynthesis) and potentially harmful for life depending on the frequency and time of exposure. Many deleterious effects are associated with exposure to high-energy electromagnetic wavelengths or ionizing radiation (including gamma, X-rays, and ultraviolet ABC (UVA, UVB, UVC) frequencies). These high frequencies can remove electrons from water and other biomolecules (i.e DNA, proteins) generating free-radicals intermediates known as Reactive Oxygen Species, ROS (i.e. superoxide anions, $\cdot O_2^-$, peroxide, $\cdot O_2^{-2}$, hydrogen peroxide, $H_2O_2$, hydroperoxyl, $HO_2^-$, hydroxyl radicals, $\cdot OH^-$ and hydroxyl ion, $OH^-$) (Ikehata and Ono, 2011; Riley, 1994). ROS are known to damage the structure and function of sensitive intracellular molecules. As a result, various biological mechanisms have evolved to limit and repair photodamage. Melanization is a conserved protection mechanism against ionizing radiation because of its optical and antioxidant properties (see below).

The role of melanin in photoprotection is widely known in the human skin, functioning as a natural sunscreen by absorbing and dissipating the photons from ionizing radiation within its structure (Wolbarsht et al., 1981). While other biological pigments are only capable of absorbing a narrow range of light frequencies, the complex-disordered structure of melanins results in the absorption of the entire UV-Visible portion of the electromagnetic spectrum (Meredith and Sarna, 2006); a similar characteristic of amorphous solid semiconductors (McGinness, 1972). Synthetic and natural melanin suspensions exhibit a distinctive monotonic optical broad-band absorption curve with maxima near the UV region, decreasing as it reaches near infrared frequencies (Meredith and Sarna, 2006; Solano, 2016; Tran et al., 2006; Watt et al., 2009). Although it is expected that all melanins should exhibit similar broad-band light absorption, the optical properties of individual fungal melanins remain largely unexplored and could differ substantially between species.

Analogous to the tanning of human skin, fungal melanogenesis is also stimulated by exposure to ionizing radiation and resulting in pigmented yeasts which are more resistant to radiotoxicity than their albino counterparts (Bultler, 1987; Durrell and Shields, 1960; Ellis and Griffiths, 1975; Gauslaa and Solhaug, 2001; Selbmann et al., 2011; Wang and Casadevall, 1994; Zhdanova et al., 1978). It is important to note that black fungi are able to survive ionizing radiation levels that are lethal to any other eukaryote (Dighton et al., 2008; Mironenko et al., 2000; Onofri et al., 2012; Selbmann et al., 2015; Zhdanova et al., 1991) which yields its own interesting implications with regards to the limits of life. The mechanism by which fungal melanin protects a cell from radiation damage likely involves a combination of several processes for absorption-dissipation of radiation energy, including:

changes in melanin’s chemical composition and structure, inelastic scattering of photons by protons or electrons (or Compton scattering), non-radiative dissipation of absorbed photons (a process that results in energy decay in the form of heat), and antioxidant or free-radical scavenging (Dadachova et al., 2008; Khajo et al., 2011; Revskaya et al., 2012).

The photoprotection capacity may differ among melanin types, radiation frequencies, and types of irradiation exposure. It is possible that photoprotection can turn into photodamage after a certain threshold of irradiation where melanin itself can produce cytotoxic radical species. Thus, under certain conditions the presence of melanin can result in increased sensitivity to radiation. For an excellent review on the photoprotective and photosensitizing functions of skin melanin refer to (Solano, 2016).

## Antioxidant

A common property of all biological pigments is their ability to accept and neutralize exogenous free radicals (McGraw, 2005). Melanins are powerful antioxidants. In fungi, they contribute to virulence by interfering with host defense factors, including neutralizing the oxidative burst of phagocytic cells (Cunha et al., 2010; Nosanchuk and Casadevall, 2003; Schnitzler et al., 1999). Fungal melanins can also protect from hypochlorite, permanganate and hydrogen peroxide (Jacobson et al., 1995). In addition to acting as powerful radical quenchers, melanins are themselves stable free radicals substances containing unpaired electrons that can respond to magnetic fields, hence its paramagnetic character. Thus, melanins can receive or donate protons depending on the conditions. The electron exchange that occurs in the melanin molecule can either oxidize or reduce metals, and fungal melanins have been used as platforms for metal nanoparticle synthesis (Apte et al., 2013).

The paramagnetic signature of melanins can be detected using electroparamagnetic spin resonance (ESR); a technique commonly used to differentiate it from other dark pigments. The free radical abundance in the melanin molecule depends on temperature, pH, humidity, and presence of metals (Enochs et al., 1993; Mostert et al., 2012). In addition, this free radical population in the melanin molecule increases upon irradiation with light in a wavelength-dependent manner such that lower wavelengths are better at inducing free radicals (Khajo et al., 2011; Sarna and Sealy, 1984). Importantly, these free electrons generated by light absorption can further be dissipated via metabolic redox chemical reactions with other organic compounds in contact to the charged melanin (Dadachova et al., 2007).

## Energy harvesting

Similar to other light-harvesting biological pigments (i.e. chlorophylls, carotenoids) microbial melanins can absorb radiation energy and transduce it onto life-nurturing chemical processes (Dadachova et al., 2007; Robertson et al., 2012; Turick et al., 2003; Turick et al., 2002) (Beatty et al., 2005; Dadachova et al., 2007; Dadachova and Casadevall, 2008; Robertson et al., 2012; Turick et al., 2003; Turick et al., 2011; Turick et al., 2002). The role of fungal melanin as an energy harvesting pigment was suspected from observations in radionuclide-contaminated environments that were colonized by melanotic microorganisms and the fungal growth attraction towards radiation sources in a process termed radiotropism

(Mironenko et al., 2000; Tugay et al., 2006). In 2007, Dadachova et al. provided experimental evidence that fungal melanin mediates an energy transduction process from light into useful metabolic energy (Dadachova et al., 2007). Using three genetically distinct fungal species the authors showed that irradiation with sub-lethal doses of gamma rays resulted in (i) an enhanced metabolic activity by 1.4 fold increase in the NADPH levels, and (ii) higher replicative rates resulting in a doubling of CFUs, paralleled by increases in dry mass and metabolic incorporation along with uptake of labeled acetate (Dadachova et al., 2007). These findings were confirmed and extended (Robertson et al., 2012). The fungal growth enhancement of melanotic fungi is dependent on the level of cell melanization and radiation dose rates (Shuryak et al., 2014). A metabolic response is not exclusive to gamma rays since irradiation of melanized fungal cells with UV-Vis radiation also results in changes in cellular ATP levels (Bryan et al., 2011). The antioxidant activity of melanin, as well as, its ability to transduce radiation energy into heat may play complementary roles in promoting fungal growth following irradiation (Bryan et al., 2011; Dadachova et al., 2007; Findly et al., 1983; Jones and Findly, 1986; Lilly et al., 1984). The capacity of melanin to harness energy from radiation is possible due to its paramagnetic and electric properties (Dadachova et al., 2007; Turick et al., 2011).

The electroconductive properties of melanin were first identified more than five decades ago (Longuet-Higgins, 1960). In the 1970's McGinness first proposed that melanin behaves as an amorphous organic semiconductor, being capable of switching between two resistive states provided enough voltage and/or heat (McGinness et al., 1974; McGinness, 1972). By definition, a semiconductor is matter capable of transporting electrical charge if delivered with sufficient potential or thermal energy (e.g. electric/magnetic field or heat, respectively).

Consistent with the semiconductor model, synthetic melanin shows a negative thermoelectric voltage under vacuum and its conductivity increases with increasing temperature (Jastrzebska et al., 1995; Osak et al., 1989). The ability of melanins to transport electric charge is significantly affected by the level of hydration, demonstrating that water is an essential part of the charge transport mechanism (Mostert et al., 2012). However, recent studies have shown that the thermoelectric effect of melanin at ambient conditions gives a positive thermoelectric voltage and the resistive behavior is not always observed, inconsistent with the classic amorphous semiconductor model (Jastrzebska et al., 1995; Jastrzebska and Wilezok, 1987; Osak et al., 1989; Trukhan et al., 1970). Melanin is now being described by experts as a porous mixed conductor, where the dominant charge it transports can change from electrons to protons as a function of increasing hydration levels (Giacomantonio, 2005). The electroconductive properties of melanin are very attractive for the development of new technologies for bioelectronics and other sustainable electronics (Albano et al., 2016; Young, 1965).

## Thermoregulation (protection against heat and cold stress)

A role of melanin in thermoregulation stems from its ability to effectively absorb solar radiation and dissipate it non-radiatively in the form of heat (Meredith and Riesz, 2004). Much like a blackbody, melanin absorbs most light and re-emits or reflects very little of the absorbed light (also known as low radiative yield) (Meredith and Riesz, 2004). The result of this energy absorption depends on a variety of parameters including the type of melanin, light frequency and the level of hydration. Although some of the energy absorbed could result in the accumulation of free radicals (Khajo et al., 2011; Sarna and Sealy, 1984), most of it is dissipated in the form of heat (Forest and Simon, 1998; McGinness and Proctor, 1973; Meredith and Riesz, 2004). This process is thought to occur by a highly efficient electron-phonon coupling; the excited electronic state of the melanin molecule relaxes via resonance with the phonons or vibrational modes in the melanin molecule (Meng and Kaxiras, 2008; Nofsinger et al., 2001; Riesz, 2007).

Melanin-mediated heat gain from sunlight is particularly important for ectothermic ('cold-blooded') organisms, which rely on the mechanisms of conduction, convection, and radiation in order to maintain temperature homeostasis. This is different from endothermic ('warm-blooded') species that maintain their body temperatures primarily from the metabolism of food. The ability of melanotic organisms to capture electromagnetic radiation and convert it to heat allows them to heat up faster and reach higher equilibrium temperatures than non-pigmented organisms. Therefore, melanization could be beneficial for an ectotherm inhabiting cold environments with low solar radiation levels, but detrimental in hot tropical climates because of the risk of over-heating. These ideas form the basis of the theory of thermal melanism of ectotherms, which can predict their geographical distribution and ecology (Clusella Trullas et al., 2007; Gates, 1980; Jong et al., 1996; Kingsolver, 1987; Watt, 1969). Examples of thermal melanism have been reported in a number of reptiles and insects (Clusella Trullas et al., 2007; Clusella-Trullas et al., 2008; Clusella-Trullas et al., 2009; Jong et al., 1996; Moreno Azocar et al., 2016; Muri et al., 2015; Tanaka, 2005). A recent study showed that turtles (*Trachemys scripta elegans*) reared in colder temperatures developed darker integument relative to those reared in warmer conditions (Rowe et al., 2016). The relevance of these ideas in maintaining temperature homeostasis in microorganisms and relevance to fungal ecology in unknown.

Although the role of melanin in fungal thermoregulation is scarce, a role in protection against heat stress was observed for *Wangiella* [*Exophiala*] *dermatitidis* (Paolo et al., 2006). Melanin-deficient mutants of *Monilinia fructicola* produced conidia that were more susceptible to high temperatures and other stressors (Rehnstrom and Free, 1996). In *C. neoformans,* melanization increased tolerance to heat and cold stress via a still unknown mechanism putatively involving quenching of heat-induce ROS or buffering heat flux (Rosas and Casadevall, 1997). Another example where melanin exhibits a role in thermoregulation is observed in symbiotic scenarios between plants and fungi, where melanized endophytes help the plant adapt to temperature changes possibly by helping dissipate heat and/or absorb ROS (Redman et al., 2002). Melanin synthesis via the tyrosinase/laccase enzymatic pathway is regulated by temperature (Jacobson and Emery, 1991; Kim et al., 2003).

## Metal binding

Given the aromatic composition of melanin polymers and the variety of hydroxyl, carboxyl, amine and phenolic functional groups present in the pigment, it is not surprising that melanin can form molecular interactions with many organic and inorganic molecules. In fact, fungal melanin can form covalent, ionic and hydrophobic bonds with proteins, polysaccharides, pesticides, drugs and other pollutants (Fogarty and Tobin, 1996; Larsson, 1993). Melanins exhibit both high binding affinity and capacity to a large number of metal ions (ie. $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Cd^{2+}$, $Mn^{2+}$, $Mn^{2+}$ and $Pb^{2+}$). This binding is dependent on pH, type of melanin and metal ion (Ben-Shachar et al., 1991; Bridelli et al., 1999; Costa et al., 2012; Fogarty and Tobin, 1996; Hong et al., 2004; Hong and Simon, 2006; Liu et al., 2004; Samokhvalov et al., 2004; Szpoganicz et al., 2002). Metal binding involves interactions with carboxyl, amine, and hydroxyl functional groups of the pigment. For instance, $Mg^{2+}$, $Ca^{2+}$, and $Zn^{2+}$, are coordinated preferentially by carboxyl, $Cu^{2+}$ by hydroxyl groups and iron (Fe3+) by hydroxyl, amine, imine and acetate groups (Costa et al., 2012; Hong et al., 2004; Hong and Simon, 2006; Samokhvalov et al., 2004). Recent electrochemical fingerprinting analysis of natural eumelanin demonstrated that its monomers may oligomerize into tetramers of porphyrin-like protomolecules, capable of coordinating multiple ions (Kim et al., 2016). The affinity of melanin to bind iron is of special interest considering its importance and abundance in biological tissues, as well as, the involvement of iron and neuromelanin in the development of pathologic changes in the human brain, as observed in diseases such as Parkinson’s disease (Double et al., 2003; Efimova et al., 2010; Schroeder and Gerber, 2014).

Metal binding modifies melanin’s physicochemical properties which may alter function. Biosorption of metals changes the paramagnetic state of melanin, increasing or decreasing the free radical populations (Buszman et al., 2006; Zdybel et al., 2015). For instance, binding of diamagnetic $Zn^{2+}$ ions results in an increase in the free radical population of melanin, but the paramagnetic $Cu^{2+}$ binding has the opposite effect (Buszman et al., 2006). Metal binding may also change the interaction of melanin with drugs and other compounds (Wrzesniok et al., 2012a; Wrzesniok et al., 2012b; Zdybel et al., 2015), presumably by blocking active centers in the melanin molecule (Wrzesniok et al., 2011).

Fungal melanins are capable of binding multiple metals (ie. $Cu^{2+}$, $Ca^{2+}$, $Mg^{2+}$, and $Zn^{2+}$) which can induce melanogenesis in some fungal species (Caesar-Tonthat et al., 1995; McDougall and Blanchette, 1996; McLean et al., 1998; Zhan et al., 2011). The metal scavenging activity of fungal melanin allows for the bioabsorption of essential metals from rocks and other environmental niches (Fogarty and Tobin, 1996; Gadd and de Rome, 1988; Rizzo et al., 1992; Zunino and Martin, 1997). Although melanin has also been proposed to protect fungi from heavy metal toxicity (Caesar-Tonthat et al., 1995; Garcia-Rivera and Casadevall, 2001; Hong et al., 2004; McDougall and Blanchette, 1996; McLean et al., 1998; Saiz-Jimenez and Shafizadeh, 1984; Tashirev et al., 2012; Zhan et al., 2011), such protection has not been observed in all cases (Frederick et al., 1999).

The remarkable ability of melanin polymers to bind metals in amounts that range from nano to micromolar amounts per mg of dry fungal mass (Fogarty and Tobin, 1996) and other toxic compounds is being exploited in bioremediation strategies of polluted waters and recovery of valuable metals ions from solution (Gadd and de Rome, 1988; Saini and Melo, 2013; Sono et al., 2012; Yan and Viraraghavan, 2003). Other applications include real-time sensitive detection of metal ions via melanin-modified sensors (Fan et al., 2014; Huang et al., 2007) and iron-melanin ingestible supplements for the treatment of iron deficiency anemia (Wang et al., 2014). The metal binding ability and metal-altered properties of fungal melanins may vary widely between species and may potentially serve in a number of biotechnology applications.

## Resistance to mechanical and chemical stress

The ability of black fungi to persist in environments of high osmotic or hydrostatic pressures (i.e. high altitudes, hypersalinity, deep waters) demonstrates the capacity of melanin to protect against various chemical and mechanical pressures. In addition to its remarkable chemical stability, melanin increases cell strengths and rigidity by depositing near the cell wall where it may crosslink with different macromolecules. Cell wall melanization also changes cell permeability and cellular turgor forces (Money et al., 1998). Melanization also protects the cell against chemical degradation such as acid hydrolysis and heavy metal toxicity (i.e. silver nitrate) (Garcia-Rivera and Casadevall, 2001). Moreover, melanization results in higher resistance to hydrolytic enzymes capable of digesting cell wall components (Bloomfield and Alexander, 1967; Kuo and Alexander, 1967; Potgieter and Alexander, 1966) as well as, osmotic stress (Kejzar et al., 2013; Kogej et al., 2007; Rehnstrom and Free, 1996; Singaravelan et al., 2008).

Melanin pigment is so resistant and stable that it can withstand the process of fossilization, whereas most organic macromolecules degrade and disappear (Lindgren et al., 2012b). Such structural stability over long periods of time also highlights the marked function of melanin as an energy transducer and suggests interesting thermodynamic properties. The ability of melanin to absorb different types of energy (i.e. radiation, free radicals) and effectively dissipate this energy could contribute to its chemical stability (Meredith and Riesz, 2004).

Although melanins are remarkably stable macromolecules they are still susceptible to biodegradation. There is evidence for melanosome degradation by hydrolytic enzymes within host cells (Borovansky and Elleder, 2003). A number of fungal species have been reported to produce manganese and/or lignin peroxidases with strong melanolytic activity (Butler and Day, 1998a; Jastrzebska et al., 1995; Liu et al., 1995; Mohorcic et al., 2007; Nagasaki, 2008; Ratto et al., 2001; White, 1958). *Aspergillus fumigatus* is able to degrade and utilize melanin as a carbon source (Luther and Lipke, 1980).

## Protection against desiccation

Fungal melanization was associated with protection and adaptation to dry conditions (Zhdanova and Pokhodenko, 1973). For example, Inhibition of melanin synthesis by the ectomycorrhizal *Cenococcum geophilum* results in increase susceptibility to osmotic stress and desiccation (Fernandez and Koide, 2013). The dark colored zones in spalted woods are caused by the melanization of certain fungal species (i.e. *Armilaria mellea*) as a survival mechanism in response to low moisture (Tudor et al., 2012). Fungal melanin may protect against desiccation by increasing the ability of cells to absorb and retain water. Melanin is known for its hygroscopic character and strong association with water which determines its electroconductive properties (Jastrzebska et al., 1995; White, 1958). Studies with synthetic and natural melanin (of non-fungal origin) have shown that melanin is associated with two forms of water: (i) one that is weakly bound and can be easily removed by drying or heating above 60 °C and (ii) another more tightly bound within the melanin structure that requires higher temperatures (above 150 °C) for its removal (Albanese et al., 1984; Bridelli et al., 1981). Since melanization can change the porosity in the cell wall (Eisenman et al., 2005; Howard et al., 1991; Jacobson and Ikeda, 2005; Kogej et al., 2007), melanin could affect the osmolite exchange making the cell more hypertonic and reduce water loss. The effect of melanisation in controlling water balance and desiccation resistance is an important determinant in the ecology of ectothermic insect species (Parkash et al., 2009).

### Cell development

Although fungal melanin does not appear to be essential for growth, it is required for normal cell development in multiple fungal species. The importance of melanin in fungal development has been primarily observed in filamentous species that, regardless of hyaline (translucent) hyphae or white mycelium, some produce melanized appressoria, sclerotia, conidia and reproductive structures (Bell and Wheeler, 1986; Butler and Day, 1998b; Henson et al., 1999). Several studies have demonstrated an association between melanin biosynthesis and healthy conidiation and germination. For example, disruption of melanin biosynthesis in the endophytic fungus *Pestalotiopsis microspore* showed little effect on vegetative growth but caused substantial alterations in the production, morphogenesis, and germination of conidia (Yu et al., 2015). These melanin-deficient conidia also appear to have altered cell wall integrity; a defect also observed with other species (Engh et al., 2007; Tsai et al., 1998; Yu et al., 2015). Altered surface morphology was reported in mutant conidia of *Aspergillus fumigatus* lacking melanin (Jahn et al., 1997; Langfelder et al., 1998; Tsai et al., 1997). Inhibition of melanin biosynthesis by tricyclazole results in defects in conidia formation and germination in *Chaetomium globosum* (Hu et al., 2012). Mutants of *Alternaria alternata* defective in melanin production produce conidia with reduced size, higher susceptibility to UV light and impaired septation of developed hyphae (Kawamura et al., 1999). A role of melanin in fungal cell development is not surprising considering the many biological functions and properties of melanin, previously discussed. The presence of melanin should impart mechanical and chemical resistance to fungal structures that may only be relevant at certain developmental stages, as fungal melanization is in turn regulated by genetic factors controlling cellular development (Engh et al., 2007; Fetzner et al., 2014; Islamovic et al., 2015; Tsai et al., 1998; Tsai et al., 1999; Upadhyay et al., 2013; Wu et al., 2012).

## Concluding remarks

This review covers the diverse functions of fungal melanins apart from its role in virulence. The structure of melanin has such complexity that it allows capture and transduction of radiation energy, thus protecting fungi against harmful forms of radiation, as well as, mediating energy use for metabolic processes. This capacity of fungal melanin to transduce radiation into metabolic energy, or radiosynthesis (Dadachova et al., 2014), suggests the presence of autotrophy in a kingdom historically classified as strictly heterotrophic. Given the contribution of fungal species to the total biomass of the Earth, this process may present significant implications for estimations of Earth’s energy balance.

The mechanisms contributing to the protective role of fungal melanin against heat and cold stress are of special interest given the extreme-unique ecological distribution of black fungi (Gostincar et al., 2012). The chelating properties of melanins towards free radicals and metals mediate protection and poses melanotic fungi as powerful tools in bioremediation and other biotechnologies (Apte et al., 2013; Dighton et al., 2008; Mahmoud, 2004; Steiner et al., 2002; Zhdanova et al., 1990).

Understanding fungal melanin function at the structural level remains a challenge given its complex nature and the paucity of techniques to analyze amorphous substances. Most, if not all, the properties of melanin described in this brief review pertain to synthetic or other natural melanins and have been broadly extrapolated to fungal melanins. The study of physicochemical properties of fungal melanin provides a favorable model to study the biological impact of melanin structure and properties in cell physiology of higher eukaryotes. Considering the large biodiversity and polyextremophilic capabilities of black fungi, as well as, the various melanin biosynthetic pathways in these species, fungal melanins may exhibit still unseen physicochemical characteristics. In this regard, X-ray diffraction studies on various fungal melanin isolates exhibit different structural profiles relative to what has been reported from synthetic melanins and neuromelanin (Casadevall et al., 2012).

When considering melanin's protective role in fungal species against a plethora of environmental stressors (i.e. hydrolytic compounds, osmotic stress, desiccation), as well as, its ancient and widespread presence in biological systems (Lindgren et al., 2012a), it is evident that melanization presents an important adaptation mechanism to Earth's climate history and eukaryotic evolution. The study of fungal melanins may provide valuable insights intp the origins and limits of Earth's life, as well as, reveal an extended potential of melanins in numerous biotechnological applications.

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## Highlights

- Black fungi are polyextremophiles.
- Fungal melanins protect against a broad range of physical and chemical environmental stressors.
- Fungal melanins function in photoprotection, antioxidation, thermoregulation, metal bioabsorption, anti-desiccation, and cell strength/development.
- Melanins play multiple ecological and biochemical functions in living organisms, consistent with a general adaptation mechanism to climate fluctuations.
- The physicochemical properties of fungal melanins may differ among species.

### Table1.

Examples of extreme environments occupied by melanotic fungi.

| | |
|---|---|
| Earth Poles (Artic and Antarctic poles) | (Tashirev et al., 2012) (Buzzini P, 2013) (Selbmann et al., 2013) |
| Tibetan Plateau (Third pole) | (Dong and Yao, 2012; Robinson, 2001) |
| Extraterrestrial conditions (i.e. International Space Station) | (Novikova et al., 2006; Onofri et al., 2008; Onofri et al., 2012) |
| Radioactive polluted areas (i.e. Chernobyl Nuclear Power Plant) | (Dighton et al., 2008; Ragon et al., 2011; Tesei et al., 2012; Vember and Zhdanova, 2001) |
| Organic and metal polluted areas | (Ban et al., 2012; Isola et al., 2013; Vicente et al., 2008; Zhan et al., 2011; Zhang et al., 2008) |
| Hypersaline waters | (Cantrell et al., 2011; Gunde-Cimerman and Zalar, 2014; Gunde-Cimermana et al., 2000; Zalar et al., 1999) |
| Acidic environments | (Baker et al., 2004) |
| Dry desserts and semi-deserts | (Durrell and Shields, 1960) (Durrell and Shields, 1960; Jiang et al., 2016) |
| Inside and surface of rocks (i.e. marbles, limestone) | (Bogomolova and Minter, 2003; Burford et al., 2003; Cappitelli et al., 2007; De Leo et al., 2003; Friedman, 1982; Gorbushina et al., 2005; Gorbushina et al., 1993; Ruibal et al., 2009; Ruibal et al., 2005; Selbmann et al., 2008; Selbmann et al., 2013; Selbmann et al., 2015; Sert et al., 2007; Staley et al., 1982; Sterflinger et al., 1999; Sterflinger and Krumbein, 1997; Wollenzien et al., 1995) (Diakumaku et al., 1995) |
| Residential washing machines and showers | (Babic et al., 2015; Thrasher et al., 2012; Zupancic et al., 2016) |
| Deep sea hydrothermal vents | (Le Calvez et al., 2009; Vandenkoornhuyse et al., 2002) |