Functions of fungal melanin beyond virulence

sciencedirect.com/science/article/abs/pii/S1749461316300641

Highlights

- •
  Melanins play multiple ecological and biochemical functions in living organisms.
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  Black fungi are polyextremophiles.
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  Fungal melanins protect against many physical and chemical environmental stressors.
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  The physicochemical properties of fungal melanins many differ among species.

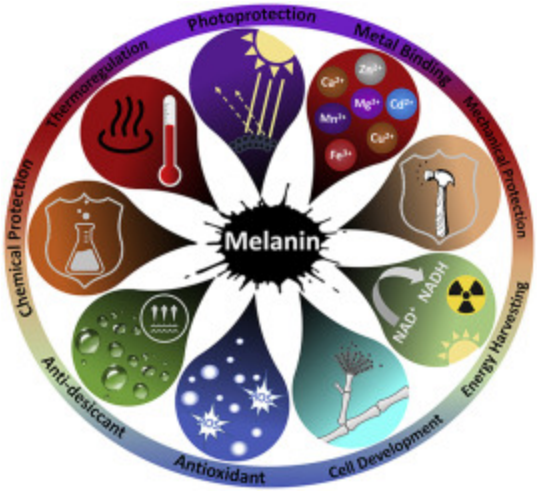
Abstract

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

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



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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, Solano, 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 π-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 nm (Bayry et al., 2014, Casadevall et al., 2012, Franzen et al., 1999, Franzen et al., 2008b, Gomez et al., 2001, Morris-Jones et al., 2003, Morris-Jones et al., 2005, 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, Ellis and Griffiths, 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, 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., 2014, Chatterjee et al., 2015, 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

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

Declaration of interest

The authors declare no competing interests.

Acknowledgements

This work was supported by the National Institutes of Health [5R01AI052733-13]. The authors are grateful to Dr. Joshua D. Nosanchuk, Dr. Laura Granell-Ortiz and Dr. Maggie P. Wear for valuable editing of the manuscript and contributions to the graphical abstract. The graphical abstract was designed and created by Dr. Wear.