The Role of Melanin in Fungal Disease

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1 Fungal Melanin Biosynthesis

Members of the fungal kingdom make many types or classes of melanins. Most fungal species produce melanins of the allomelanin or eumelanin classes, canonically from the polymerization of 1,8-dihydroxynaphthalene (1,8-DHN) and derivatives of L-3,4-dihydroxyphenylalanine (L-DOPA) and other catecholamines, respectively (Eisenman and Casadevall 2012). Other fungal melanin subtypes have been reported, including pyomelanin production from homogentisate (HGA) by *Aspergillus fumigatus* (Schmaler-Ripcke et al. 2009) and GHB-melanin from the precursor glutaminyl-hydroxy-benzene (GHB) by *Agaricus biosporus* (Weijn et al. 2013). The production of a novel 5-deoxybostrycoidin-based melanin in *Fusarium* species (Frandsen et al. 2016), and an aspulvinone E-based melanin (Asp-melanin) by *Aspergillus terreus* (Geib et al. 2016) have been reported. However, further study of these novel compounds is necessary. Here, we describe the reported mechanisms of fungal biosynthesis for the three most prevalent melanin subtypes.

Aspergillus spp. as well as *W. dermatitidis* and *Sporothrix schenckii,* produce allomelanins, also known as DHN melanins (Gow et al. 2017). These melanins are black or brown, are typically attached to the inner side of fungal cell walls (Tran-Ly et al. 2020), and are produced from the polymerization of 1,8-dihydroxynaphthalene (DHN) (Britton 1983). As such, they do not contain nitrogen. DHN is produced from the polyketide pathway, which begins with either Acetyl-CoA or Malonyl-CoA.

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These precursors undergo decarboxylative condensation via polyketide synthase to form 1,3,6,8-tetrahydroxynaphthalene (THN) (Singh et al. 2021). The reduction of THN by THN reductase to form scytalone is followed by two rounds of dehydration to ultimately produce the monomer DHN (Singh et al. 2021). Fungal mutants with mutations of the gene encoding polyketide synthase (pksP or alb1 for "Albino 1") produce albino conidia in the absence of exogenous scytalone (Tanguay et al. 2006). In *Aspergillus fumigatus*, the genes for all six enzymes involved in DHN-melanin synthesis are encoded by a 19 kb gene cluster on the second chromosome (Tsai et al. 1999).

Other fungi, including *Cryptococcus neoformans, Candida auris,* and *Paracoccidioides brasiliensis*, produce nitrogen-containing eumelanin from catecholamine-based derivatives such as L-DOPA (Eisenman et al. 2007; Gómez et al. 2001). Eumelanin, a typically black-brown pigment, is formed by the polymerization of indoles. These indoles are canonically formed through a multi-step biosynthetic pathway, beginning with the oxidization of catecholamines into reactive quinones. These quinones can then cyclize via an intramolecular nucleophilic attack, after which their spontaneous oxidization and tautomerization form the necessary indoles (Eisenman and Casadevall 2012). While this pathway can occur spontaneously through autopolymerization, in most fungal species the first conversion to quinone is catalyzed by laccases (EC 1.10.3.2) or phenol oxidases (EC.1.14.18.1 and EC.1.10.3.1). Further, for fungal species that produce the phenol oxidase tyrosinase, the enzyme can catalyze both the oxidation of catecholamines to quinone as well as an additional precursor step converting L-tyrosine into the catecholamine L-DOPA, allowing for the production of eumelanin from an endogenous amino acid (Smith and Casadevall 2019).

In addition to the biosynthetic L-DOPA pathway originating from L-tyrosine, melanin can also be produced by the oxidation and subsequent polymerization of HGA, an intermediate in the degradation pathway of L-tyrosine. Mutation of the enzyme homogentisate dioxygenase that normally catalyzes the conversion of HGA to maleylacetoacetate in this pathway causes a rare human genetic disorder called alkaptonuria (La Du et al. 1958), characterized by a build-up of HGA and production of alkaptomelanin. Its microbial counterpart, pyomelanin, was first identified in the bacterium, *Pseudomonas aeruginosa* (Yabuuchi and Ohyama 1972), and has been discovered subsequently in several fungal species, such as *Apergillus fumigatus*, *Sporothrix* spp., and *Histoplasma capsulatum* (Schmaler-Ripcke et al. 2009; Almeida-Paes et al. 2012, 2018). For fungal species that produce melanin by multiple pathways, pyomelanin is identified by its unique susceptibility to sulcotrione, a specific inhibitor of 4-hydroxyphenylpyruvic acid dioxygenase (HppD), the enzyme that converts 4-hydroxyphenylpyruvic acid to HGA (Lorquin et al. 2022). At least one instance of direct competition between pathways has been noted wherein pyomelanin synthesis by *Alternaria alternata* supplants the more canonical DHN-melanin pathway through down-regulation of CmrA, the key transcriptional activator of DHN-melanin synthesis genes (Fernandes et al. 2021). The upregulation of HppD that occurs upon the transition from the filamentous to parasitic yeast form of several fungal species including *H. capsulatum,*

Paracoccidioides brasiliensis, and *Talaromyces marneffei* (Nunes et al. 2005; Boyce et al. 2015; Hwang et al. 2003) argues that pyomelanin may play a key role in fungal virulence.

2 Melanin Structure and Localization

The elucidation of melanin's exact chemical structure has been hampered due to the insoluble and heterogeneous character (e.g., variable starting monomers) of this biomaterial (Prota 1988). Any attempt at solubilization disrupts its structure and adds complexity to its structural analysis, the macroscale assembly of melanin is amorphous ("disordered") and thus not approachable with standard methods of structure determination such as X-ray crystallography. It involves supramolecular interactions within melanin and between melanin and other surrounding components. In the past three decades, the use of alternative and non-destructive spectroscopic methodologies (Casadevall et al. 2012; Chatterjee et al. 2012, 2014, 2015; Chrissian et al. 2020a–c; Baker et al. 2021; Camacho et al. 2017) along with high-resolution transmission electron microscopy (TEM) (Eisenman et al. 2005, 2009; Walker et al. 2010; Wolf et al. 2014; Franzen et al. 2008; Alviano et al. 1991; Almeida-Paes et al. 2017; Romero-Martinez et al. 2000; Freitas et al. 2019) and proteomics (Camacho et al. 2019; Almeida-Paes et al. 2020) have provided tremendous insights about the complex hierarchical assembly structure of fungal melanins.

In most fungal species, melanins are mainly deposited in layers within the cell wall displaying variations in their distribution (e.g., in inner or outer regions) (Eisenman et al. 2005; Walker et al. 2010; Franzen et al. 2008; Romero-Martinez et al. 2000; Nosanchuk and Casadevall 2003a; San-Blas et al. 1996; Bayry et al. 2014). However, given that melanin synthesis starts intracellularly, it is also detected in cytoplasmic deposits within membrane-enclosed compartments known as melanosomes as well as along the plasma membrane (Freitas et al. 2019; Camacho et al. 2019; San-Blas et al. 1996). The distribution and maintenance of melanin within the cell wall depend on covalent and non-covalent interactions with other cell wall components such as chitin, chitosan, glucan, and lipids.

The molecular organizational structure of melanins consists of locally-ordered oligomer sheets that form planar stacks with variable stacking distances due to differences in the chemical composition (Büngeler et al. 2017). In agreement with this model, studies using X-ray powder diffraction demonstrated that fungal melanins isolated from *C. neoformans, Wangiella dermatitidis*, *Aspergillus niger,* and *Coprinus comatus* conserved this basic stack sheet structure, with stacking distances between the melanin layers ranging from 3.46 to 4.39 Å, which may serve as a key parameter for further melanin categorization (Casadevall et al. 2012). While the supramolecular "disordered" structure is a consequence of the planar structures exhibiting diverse orientations to one another stabilized by hydrogen bonding, cation-π, and van der Wall interactions (Hong et al. 2018).

The most studied system of fungal melanin supramolecular architecture is that of *C. neoformans,* where complementary approaches have been used to elucidate its

cell wall building block unit (Camacho et al. 2019). That work investigated melanin hierarchical buildup from "melanin ghosts" (melanin carcasses from a hollow cell after acid exposure) and from structures released in the culture supernatant. Two main structures were identified: (1) Melanosomes; and (2) Melanin granules. The **melanosomes**, the structural unit of the cell-wall melanin that corresponds to ~30 nm in diameter smooth nanospheres. These are visualized by TEM within multivesicular bodies (MVBs) and vesicles in the cell cytoplasm or exposed in the cell wall after extended acid-hydrolysis of melanin ghosts. Similar melanosomes in the structure had been reported for other natural eumelanins (Xiao et al. 2018; Franzen et al. 2006). The **melanin granules** are aggregated melanosomes measuring from 40 to 200 nm in diameter, which result from the accumulation and crosslinking of melanosomes among each other and to surrounding non-pigmented components with different degrees of melanization. These are found intracellularly, within the cell wall, or in the extracellular media.

2.1 Cell-Wall Associated Melanin

Melanin granules can be arranged in layers within the cell wall (Chrissian et al. 2020a; Eisenman et al. 2005; Franzen et al. 2008; Romero-Martinez et al. 2000) or clustered on the cell wall surface (Walker et al. 2010; Romero-Martinez et al. 2000; Bayry et al. 2014). In *Cryptococcus* species and *Candida albicans* cell-wall chitin or its deacetylated form, chitosan, plays a key role in the melanin accumulation and distribution within the cell wall (Chrissian et al. 2020a; Camacho et al. 2017; Walker et al. 2010). Disruption of the chitin synthesis in *C. neoformans* (Tsirilakis et al. 2012) results in a leaky-melanin phenotype where melanin is not retained within the cell wall and is released to the extracellular medium. A similar leaky phenotype is also observed upon binding of cell wall dyes that interfere with melanin deposition (Perez-Dulzaides et al. 2018). Aliphatic groups identified as triglycerides (TGs) within fungal melanins are associated with their synthesis within vesicles (Eisenman et al. 2009; Zhong et al. 2008; Rodrigues et al. 2007) and cell-wall remodeling processes during budding (Nosanchuk and Casadevall 2003a). More recently, ss-NMR studies determined that melanized cells of *C. neoformans* were not only associated with TGs but also with sterol esters (SE) and polyisoprenoids. These lipids were also found in non-melanized cells but given that TGs and SEs are the typical cargo of lipid droplets, it may be possible that these organelles are involved in *C. neoformans* melanin synthesis (Chrissian et al. 2020c).

2.2 Secreted Melanin

During fungal growth and cell replication, melanized fungal cells have to remodel their cell wall during budding and morphological transitions such as making hyphae. To allow cellular budding, the local cell-wall remodeling might be driven by

secreted enzymes (peptidase, chitinases, and glucanases) (Geddes et al. 2015) that break melanin linkages to cell-wall components. In *C. neoformans*, using isopycnic gradient sedimentation, detached and secreted melanin granules in the culture supernatant were isolated and analyzed for proteins (Camacho et al. 2019). This study identified four proteins (Qsp1, Cig1, Blp1, and CNAG_05313) that may play important roles in the fungal melanogenesis and adaptation/survival of the fungus inside the host.

3 Role of Fungal Melanin in Human Disease

While mammalian endothermy, among other factors such as advanced immunity, protects against many fungal species, some species have evolved to be pathogenic in humans (Köhler, Hube, et al.). A concern particularly in those who are immunocompromised, the continued evolution of fungal virulence represents a growing threat to global health. While many fungal infections are superficial and mild, some may evolve into severe diseases, especially in the aforementioned immunocompromised hosts. For example, while *Candida albicans* is commonly a harmless colonizer of human mucous membranes, it can lead to fatal systemic candidemia in those with neutropenia. Meanwhile, infections with *Cryptococcus neoformans* or *C. gattii* can cause disseminated cryptococcosis in both healthy and immunocompromised adults, often leading to subacute meningoencephalitis (Köhler, Casadevall, et al.). To be pathogenic in humans, these fungi must evolve to be able to withstand the human febrile temperatures of 38–39 °C and resist the efforts of the immune system targeting fungal cells. Notably, melanin produced by the pathogens *Cryptococcus neoformans* and *Monilina fructicola* has been shown to confer thermotolerance, representing a potential role for fungal melanin in this evasion of mammalian endothermy (Cordero and Casadevall), especially when exogenous melanization substrates are available in the extracellular environment, such as in infection of the substantia nigra. Further work is needed to better elucidate the contributions of melanin's conferral of thermotolerance to the development of fungal pathogenesis; however, other roles for melanin in fungal virulence are well described.

3.1 Cell-Host Interaction

Among the innate immune receptors, host pattern recognition receptors (PRRs) can be divided into two groups: secreted receptors and transmembrane signal-transducing receptors (Mortaz et al. 2017; Brubaker et al. 2015; Latgé 2020). Pathogen-associated molecular patterns (PAMPs) are highly conserved molecular structures found in some pathogenic microorganisms and are known to be critical in

initiating innate immune responses and inducing and directing subsequent adaptive immunity (Latgé 2020; Kurup and Tarleton 2013).

Most host cells express at least one type of cellular PRRs. PRRs can be divided into five different types: Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), C-type lectin receptors (CLRs), and absent in melanoma-2 (AIM2)-like receptors (ALRs). Among them, TLRs and CLRs have been extensively studied in the context of fungal infection and also play central roles in antifungal immunity (Mortaz et al. 2017; Brubaker et al. 2015).

Several immunologically fungal ligands have been described as PRRs, including melanin 2,3. A C-type lectin receptor, called Melanin sensing C-type Lectin receptor (MelLec/CLEC1A), was shown to play an essential role in antifungal immunity through recognition of the naphthalene-diol unit of 1,8- dihydroxynaphthalene (DHN)-melanin. However, MelLec is not able to bind to DOPA-melanin, produced by other fungal pathogens, including Cryptococcus sp. (Smith and Casadevall 2019; Stappers et al. 2018). In humans, this receptor is expressed by endothelial cells and leukocytes, including monocytes, dendritic cells, and granulocytes, but not by lymphocytes (Sattler et al. 2012). In aspergillosis experimental infection, MelLec was required for early leukocyte recruitment in the lungs (Stappers et al. 2018). In summary, MelLec is a receptor recognizing an immunologically active component commonly found in fungi and plays an essential role in protective antifungal immunity in both mice and humans, showing the importance of fungal melanin as PAMPs and how it can be sensed and recognized by immune host cells, activating the development of the appropriate immune response (Stappers et al. 2018).

3.2 Mechanisms of Resistance to Human Host Immune Factors

3.2.1 Oxidative Stress

Two key features, namely a negative charge and a stable free radical population, are shared among the characteristics of melanins produced by fungi (Smith and Casadevall 2019). These properties confer upon fungal melanins the ability to reduce oxidizing free radicals, highly reactive molecular species with one or more unpaired electrons (Lobo et al. 2010). In the context of fungal infections, free radicals produced by host immune cells during oxidative bursts can be absorbed and neutralized by melanin in the fungal cell wall before they can enter the cell and elicit cytotoxic damage (Nosanchuk and Casadevall 2003b). For example, DOPA-derived eumelanin produced by *Cryptococcus neoformans* imparts a survival advantage to cells treated with oxidative reactants *in vitro* (Jacobson and Tinnell 1993; Wang and Casadevall 1994a) or during phagocytosis by macrophages (Wang et al. 1995). Enhanced survival of melanized cryptococcal cells inside macrophages is expected to contribute to virulence by promoting dissemination from the lungs to the brain

through a Trojan horse mechanism (Liu et al. 2012) and a recent study reporting a survival advantage for melanized compared to non-melanized *C. neoformans* cells in a mouse model of cryptococcal infection supports this hypothesis (Baker and Casadevall 2023). Other forms of melanin have been shown to protect fungal species from the types of free radicals released by host macrophages during phagocytosis, including DHN-melanin in *Sprorothrix schenkii* and *Fonsecaea pedrosoi* (Romero-Martinez et al. 2000; Cunha et al. 2010) and both DHN and pyomelanin in *Aspergillus fumigatus* (Schmaler-Ripcke et al. 2009; Jahn et al. 1997). Thus, melanization is a widespread adaptation that permits prolonged survival of infective fungal cells within host phagolysosomes thereby increasing their propensity to cause disease.

3.2.2 Melanin Interference with Antifungal Drug Activity

Melanin also contributes to fungal virulence through the sequestration of antifungal drugs. Melanization of *C. neoformans* and *H. capsulatum* reduces the potency of the polyene amphotericin B and the echinocandin caspofungin (van Duin et al. 2002). These compounds have also been observed to change the elemental composition of fungal melanin after incubation *in vitro*, suggesting a mechanism of direct binding and sequestration (Nosanchuk and Casadevall 2006). Melanized *P. brasiliensis* cells have also demonstrated reduced susceptibility to amphotericin B, and, in contrast to that observed in *C. neoformans*, also manifested reduced susceptibility to azoles like fluconazole, ketoconazole, itraconazole, and sulfamethoxazole (Gómez et al. 2001). Notably, the direct sequestration of azole drugs by melanin has yet to be demonstrated. However, fungal melanins have been shown to bind to a variety of drug types beyond just the antifungals, with binding efficacies approaching other known absorbers like medicinal activated charcoal (Bridelli et al. 2006).

Further, treatment with the DHN-melanin synthesis inhibitor tricyclazole increased the potency of terbinafine in *Sporothrix brasiliensis* and *Sporothrix schenckii* species (Almeida-Paes et al. 2016). And, interestingly, antifungal drug treatment has also been shown to increase rates of DHN-melanin synthesis in *A. infectoria*, affecting the compound's deposition in the cell wall (Fernandes et al. 2015). Electron micrographs of melanized *C. neoformans* cells have demonstrated melanin deposition in the cell wall, providing support for extracellular drug capture by melanins (Eisenman et al. 2005). Melanization makes the cell wall less porous to amphotericin-containing liposomes suggesting another mechanism by which this pigment can reduce fungal susceptibility to this antifungal drug (Walker et al. 2018). Taken together, the role of melanin in antifungal drug resistance and uptake has been robustly established.

3.2.3 Immune Evasion

The extracellular localization of fungal melanin also contributes to its role in the evasion of host immunosurveillance. Melanin in *Aspergillus fumigatus* has been

shown to mask pathogen-associated molecular patterns (PAMPs) like mannans and β-glucan from the immune recognition, significantly attenuating the observed cytokine response (Liu et al. 2021). Likewise, melanin from *A. nidulans* was shown to have an anti-inflammatory effect, decreasing the production of nitric oxide and TNF-α in stimulated macrophages (Gonçalves et al. 2013). In *C. neoformans,* phagocytosis of melanized cells was observed to be lessened compared to those unable to form melanin (Mednick et al. 2005). In addition, Rosas et al. showed that the injection of *C. neoformans* melanin isolated particles could induce granuloma formation in mice; interestingly, the granulomas and the latent infection commonly associated with them are observed in pathogens capable of melanization (Nosanchuk and Casadevall 2006; Rosas et al. 2002). In addition to the protection conferred by its immunomodulatory and ROS scavenging roles, melanin has also been shown to be protective against enzymatic degradation and secreted antimicrobial peptides like defensins, likely due to its negative charge and promiscuous binding affinity (Rosas and Casadevall 2001). Broadly, melanin knockout has been shown to decrease fungal virulence (McClelland et al. 2006), underscoring its importance in fungal pathogenesis and human disease.

4 Interactions Between Fungal Melanins and Insect Hosts

Insect hosts provide an interesting context for melanized fungi. Insects, like most arthropods, produce their own melanin as an important part of their immune response to microbes, including fungi (González-Santoyo and Córdoba-Aguilar 2012). Insect immune melanization is produced by the oxidation of catecholamines in the hemolymph by activated phenoloxidases, resulting in the formation of DOPA melanins. The melanization reaction is believed to kill the microbes through the oxidative and toxic intermediates produced by the melanization reaction (Zhao et al. 2011). The interactions between fungal melanins and insect melanins have not been extensively studied, but current evidence in the literature indicates that fungal melanins are not advantageous for fungal survival and limit infection within insects. In contrast to mammalian and plant fungal pathogens, many entomopathogenic fungi—or fungi that infect insects—do not produce melanin pigment, including *Metarhizium anisopliae* and *Beauveria bassiana* (Lu et al. 2021; Fang et al. 2010). Since insect melanins are used for immunity and wound healing, fungal melanins may act as damage-associated molecular patterns (DAMPs), which in turn would activate more immune and wound-healing responses as seen in *in vitro* studies investigating melanization of insect hemolymph (Smith et al. 2022).

During infections of *Galleria mellonella* wax moth larvae, pigmentation mutants of *Aspergillus fumigatus* are more virulent than their wild-type melanized counterparts (Jackson et al. 2009). Conversely, melanin-deficient *A. fumigatus* mutants (*alb1Δ)* were less virulent during oral and topical infection of *Drosophila melanogaster* fruit flies deficient in *Toll,* an immune gene responsible for recognizing microbes (Lionakis et al. 2005). While these findings appear contradictory, if the

fungal melanin activates an effective immune response via *Toll* signaling, then the *Toll*-deficient *D. melanogaster* mutants would not reveal an enhanced virulence phenotype of albino fungal mutants. Non-melanin-producing mutants of *Fonseca monophora* are more virulent than the melanized counterparts in *G. mellonella* larvae (Liu et al. 2019). Lastly, cultures from wildtype non-melanized *Cryptococcus neoformans* are also more virulent than the wildtype melanized cultures in *G. mellonella* (Eisenman et al. 2014). The melanized cells induced larger inflammatory nodules, indicating that melanin can activate inflammation and immune reactions in the larvae (Eisenman et al. 2014). These nodules are often sites of the insect's immune melanization reaction and are key in controlling infection (Dubovskiy et al. 2016). Additional evidence shows that the melanin-producing enzyme laccase from *C. neoformans* can activate the insect's melanization response (Smith et al. 2022), although the laccase-null *lac1Δ* mutant is hypovirulent in *G. mellonella* infections, possibly due to other non-fungal melanin related roles (Lu et al. 2021; Mylonakis et al. 2005). On the other hand, a strain of the entomopathogenic fungus *M. anisopliae* that was genetically modified to produce melanin resulted in mutants that had enhanced virulence and enhanced stress tolerance overall in a wide variety of insect pests (Tseng et al. 2011, 2014).

In studies investigating the correlation between virulence factors, fungal properties, and survival of insect hosts, the role of fungal melanin is less clear. Survival of *D. melanogaster* positively correlated to the degree of melanization of *Cryptococcus spp.* isolates, indicating that degree of fungal melanization is maladaptive in the case of infections of insect hosts (Thompson et al. 2014), while fungal melanization from *Cryptococcus gattii* isolates was correlated with increased virulence in *G. mellonella* (Firacative et al. 2014). These studies only provide correlations between virulence and melanization, which may be influenced by many other virulence factors and conditions, including capsule size and fungal growth rate. Additional experiments studying the nuanced and mechanistic interactions between fungal melanin and the insect immune response need to be done.

5 Role of Fungal Melanin in Plant Infections

The melanin in melanized fungi can also play a surprisingly crucial role beyond human and insect hosts. Black fungal pathogens have a significant impact on agriculture globally. One example is the species *Colletotrichum.* This species predominantly causes anthracnose disease, red rot, crown rot, and brown blotch (Cannon et al. 2012). The fungi are so expansive that it affects: papaya, citrus, strawberry, tomato, corn, alfalfa, pepper, legumes, radish, coffee, and sorghum plants to name a few (Dean et al. 2012). The melanin in melanized fungi can also play a surprisingly crucial role beyond human and insect hosts. More specifically, melanized fungi use melanin to create and maintain high turgor pressures in the appressorium while inserting themselves into the plant hosts, absorbing essential

minerals which function as a reservoir for the fungi, and preventing loss of glucose (Nosanchuk and Casadevall 2003b; Butler et al. 2001).

The melanin produced by fungal plant pathogens plays a significant role in the colonization of the plant host. To colonize a plant host, fungi produce appressoria, or tiny hyphal cell formations containing glycerol, which help create enough turgor pressure to penetrate the epidermal cells of plants. Specifically, melanized appressoria are comparatively advantageous to non-melanized appressoria in terms of generating and maintaining sufficient turgor pressure to invade the plant (de Jong et al. 1997). Melanized fungi more effectively prevent diffusion of glycerol which retains a higher turgor pressure necessary for the degradation of the cuticle (de Jong et al. 1997). A notable example of this phenomenon is the fungus *Magnaporthe grisea* which utilizes melanin to invade rice plants and result in rice blast disease (Howard and Valent 1996). When the same *M. grisea* is treated with a tricyclazole, a reagent that prevents the synthesis of melanin, or an albino mutant of *M. grisea* is used, the appressoria are unable to generate sufficient turgor pressure (Howard and Valent 1996).

The role of melanin in melanized fungi is not limited to just host-invasion processes; it can also play a defensive role. Melanin can aid the survival of melanized fungi even while in a dormant state (Butler et al. 2001). Fungi which produce melanized sclerotia, a bundle of hyphae, are far more resistant to chemical attacks (Butler et al. 2001); this is incredibly important for resistance against fungicides as well. For fungi that do not produce sclerotia, melanin still plays a role in protecting the fungi (Butler et al. 2001). This is evident, for example, in mutant versions of fungi *G. Graminis* which are more susceptible to ultraviolet radiation, lytic enzymes, and some antimicrobial agents (Frederick et al. 1999).

Melanotic fungi pose clear offensive and defensive advantages over their non-melanized counterparts. More specifically, melanized fungi are more effectively able to invade plants, maintain nutrients, and protect themselves against an array of chemical, radioactive, and other physical threats.

6 Fungal Melanin as a Target for Antimicrobial Therapies

Fungal melanin is of particular consideration in the development of antimicrobial therapies due to its roles both in fungal virulence and antimicrobial resistance. Melanized fungi can exhibit decreased susceptibility and enhanced resistance to antifungal medications. For example, while amphotericin B (AmB) is often effective against non-melanized *C. neoformans* in vitro (Wang and Casadevall 1994b), it likely acts by causing an increase in ROS (Sangalli-Leite et al. 2011). Due to melanin's antioxidant properties, *C. neoformans* grown with L-Dopa exhibited significantly enhanced survival against AmB at concentrations up to 0.3 μg/ml (Wang and Casadevall 1994b). In vivo melanization of *C. neoformans* may hinder amphotericin B's fungicidal action in clinical settings (Nosanchuk and Casadevall 2006).

Past research has suggested that inhibition of melanization can be an effective infection control strategy. When Alviano et al. collected sera from patients with chromoblastomycosis, purified melanin-binding antibodies were shown to opsonize melanotic *Fonsecaea pedrosoi* conidia in vitro. In addition, treating mice with monoclonal antibodies (mAbs) to melanin significantly improved survival against *C. neoformans* infection (Rosas et al. 2001). In addition, mice administered mAbs experienced significantly less *Cryptococcal* growth both in the lungs and the brain (Rosas et al. 2001).

Glyphosate, a glycine analog, and a component of the herbicide Roundup interfere with the shikimate pathway, which is used by many organisms for the synthesis of aromatic amino acids. Because melanin is synthesized from phenolic compounds, glyphosate can also interfere with melanin synthesis in fungal pathogens such as *C. neoformans*. Nosanchuk et al. demonstrated that mice infected with *C. neoformans* and administered glyphosate displayed prolonged survival and decreased *C. neoformans* melanization (Nosanchuk et al. 2001). On the other hand, in organisms that rely on melanin production for the immune defense such as insects (See Sect. 4), glyphosate increases host susceptibility to microbial infection (Smith et al. 2021). In total, the body of existing research points to a need for further studies into the use of inhibiting melanin synthesis and/or activity for antimicrobial purposes.

7 Concluding Remarks

Melanin is a multifunctional polymer that has varied roles in fungal pathogenesis ranging from interfering with the immune system in animals to promoting turgor pressure for plant-invasive fungi to protecting fungal cells from antifungal drugs. Melanin functions in virulence stand apart from the other mechanisms by which this pigment promotes fungal survival in the environment (Cordero and Casadevall 2017) such as conferring protection against amoeba predators (Steenbergen et al. 2001), ultraviolet light (Wang and Casadevall 1994c) and cellular mechanical strength (Mattoon et al. 2023) and promoting the capture of electromagnetic energy for growth (Dadachova et al. 2007) and thermal regulation (Cordero et al. 2018). Interference with melanization is a potential therapeutic strategy that is currently unexploited in drug development. Despite all we know about this enigmatic polymer there are major unresolved problems in the biology of melanin ranging from uncertainty in its structure to the mechanisms by which it is assembled in the cell wall and rearranged during budding and cellular morphological transitions. The study of melanization and its effects on virulence are exciting and productive frontiers in fungal pathogenesis.

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