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REVIEW

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Melanin in fungi: advances in structure, biosynthesis, regulation, and metabolic engineering

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Abstract

Fungi can synthesize a diverse range of melanins with appropriate physicochemical and biological characteristics for numerous applications in health, environmental protection, energy, and industry. Gaining deeper insights into the chemical structures, biosynthetic pathways, and regulatory mechanisms of fungal melanin would establish a basis for metabolic engineering approaches, aimed at enhancing production efficiency and creating custom-designed melanin with desirable material properties. Due to growing interest in their beneficial effects and applications, research on the structure, biosynthesis, and regulation of fungal melanin has significantly advanced. This review highlighted recent progress in fungal melanin production and applications, concentrating on structure, biosynthesis, and regulatory networks, and suggested how an improved understanding of melanin biosynthesis could enable efficient production for future applications.

Keywords Melanin, Structure elucidation, Regulatory network, Production, Bioengineering

Background

An Italian scientist, Bartolomeo Bizio (1825), extracted a pure black matter from the cuttlefish *Sepia*'s ink and named it "melaina". The term was later modified to "melanos" by a Swedish chemist Berzelius in 1840, describing only a dark-colored pigment [1]. However, the popular term "melanin" refers to polymeric pigments colored in tone from light to dark. It is synthesized through polymerization of highly ordered phenolic or indolic compounds and is present in animals, plants, fungi, and bacteria [2]. Melanin is a negatively charged, hydrophobic, and insoluble pigment with a high molecular weight, resistant to dissolution in both aqueous and organic solvents. It possesses a wide range of distinctive physicochemical characteristics, comprising broadband optical absorption, paramagnetism, hydrophilic, and profound structural stability. Because of these intrinsic properties, melanin can be an important multifunctional biomaterial. Melanotic fungi, particularly *ascomycetous* and *basidiomycetous*, can produce a wide range of melanins, remarkably attracting scholars' attention in recent years [3]. Fungi-produced melanin has been linked to fitness and survival in harsh environments, including extreme temperatures, oxidative stresses, drought, toxic heavy metals, and ionizing radiation [4].

Melanin was previously extracted from cephalopod ink [5] or chemically synthesized [6]. However, both approaches are expensive and cause environmental pollution. Microbial-based bio-production has received scientists' attention due to its eco-friendliness, high manipulability, and widespread availability [7]. In

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contrast to other microorganisms, fungi are notable chassic organisms for melanin synthesis because of their remarkable metabolic versatility (a wide range of temperature, pH, salinity, and nutrition conditions), singular localization of melanin synthesis products (in the cell wall or secreted into extracellular space), cost-effective culture substrates, and established fermentation protocols. These advantageous features make fungi excellent candidates for melanin production [8, 9].

Melanogenic fungi can be isolated and cultured successfully on synthetic substrates. Addition of melanin precursor compounds and metal ion inducers promotes the scale-up production of natural melanin. A promising approach for increasing melanin yield involves optimizing fermentation conditions and manipulating genes involved in melanin synthesis via bioengineering technology. In recent years, there has been a surge of extensive research efforts devoted to elucidate structural properties of melanin, accompanied by the discovery of a plethora of novel regulatory factors associated with melanin synthesis in fungi [10–13]. These groundbreaking discoveries have unquestionably advanced our understanding of the complex structure of natural melanin, as well as the intricate regulatory network that governs melanin production in fungi. This review aimed to discuss the structure of fungal melanin and describe the mechanisms of melanin biosynthesis and regulation network, thereby inspiring the advancement of industrial melanin production.

Structure and properties of fungal melanin

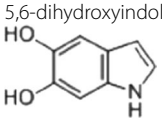
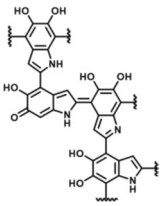
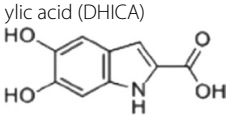
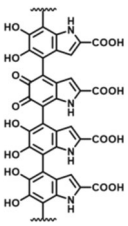
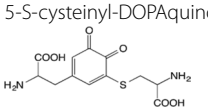
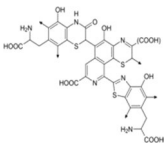
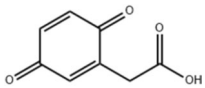
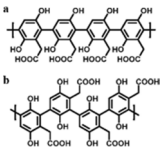
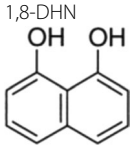
Melanin combined with proteins, polysaccharides, lipids, and other macromolecules in the fungal cell wall, can preserve the spherical shape of cells [14]. Due to the heterogeneity of melanin, its insolubility in water or organic solvents, and the harsh chemical treatments required to separate it from fungal cells, the structural elaboration of the melanin molecule has been difficult. Whereas achieving high-resolution structural elucidation of melanin molecules remains challenging, significant progress has been made in unraveling enigmatic architecture of this natural biopolymer. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) have been utilized to detect the melanin surface morphology, whereas X-ray photoelectron spectroscopy (XPS), ultraviolet (UV)-visible spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, electron paramagnetic resonance (EPR) spectroscopy, solid-state nuclear magnetic resonance (NMR) spectroscopy, and high-performance liquid chromatography (HPLC) can elucidate the photophysical properties of melanin.

Melanins are black nano-particles arranged in concentric layers of fungal cell wall [15]. The internal structure of these "melanin ghosts" is characterized by a hollow morphology, accompanied by the presence of multiple irregular 1–30 nm pores [16, 17]. The presence of a high C/H ratio and absorption peak in the visible region may confirm the existence of an indole-based aromatic core in natural eumelanin [18, 19]. The condensed molecular formula ($[C_{18}(OR)_3H_7O_4N_2]n$) was first reported by Sun et al. [20]. The structure of each melanin monomer depends on its unique synthetic precursors, thereby inducing variations in its properties across different melanin types (Table 1). Specific inhibitors, element analysis, detection of specific degradation products, and quantifying a set of photochemical properties are effective strategies to determine the melanin type produced by various fungal species. Element analysis refers to the assessment of primary elements, such as carbon, hydrogen, nitrogen, sulfur, and oxygen, which vary across melanin types due to differences in precursor molecules.

Eumelanin and pheomelanin are both classified as DOPA-melanins. Eumelanin is derived from indole precursors that are formed through the oxidation of L-dopa or L-tyrosine, resulting in sulfur-free structures. In contrast, pheomelanin is a cysteinyl conjugate produced from dopa, containing 9–12% sulfur in its structure, which enables it to dissolve in alkaline media [30]. Eumelanin derived from either 5,6-dihydroxyindole (DHI) or 5,6-dihydroxyindole-2-carboxylic acid (DHICA) exhibits distinct light absorption and paramagnetic properties. DHI-based eumelanin functions as a relatively weak H-atom donor with limited free radical scavenging capacity. In comparison, DHICA-based eumelanin has enhanced antioxidant capabilities due to additional carboxyl groups, although this improvement is associated with significantly reduced visible light absorption and a weaker paramagnetic response [30]. Due to the presence of proton transfer-coupled ring-opening reactions within its structure, pheomelanin is phototoxic and can easily lead to the production of reactive oxygen species (ROS) [23, 31]. In contrast, eumelanin contains a carbon-centered radical besides the extra semiquinone free radicals, whereas the radicals of pheomelanin are typically localized to N atoms and coupled to a π system [25, 32]. In some cases, pheomelanin potentially acts as a molecular framework in promoting eumelanin polymerization. The indole rings, C=O, $-(CH_2)n$, $-CH_nO$, –COOH, or –CONH in eumelanin confer specific light absorption characteristics distinguishing it from other pigments [33, 34].

Of note, pyomelanin is a nitrogen-free melanin generated through the polymerization of pyrrole-2,3,5-tricarboxylic acid and comprises 2-acetyl-1,4-benzoquinone units. The molecular mass of pyomelanin is comparatively the smallest among all pigments, typically ranging

Table 1 Characteristics of different types of melanin

Type of melanin	Precursor of each melanin type	Monomer of each melanin type	Technology for melanin structure elucidation	Properties of melanin type	References
Eumelanin	5,6-dihydroxyindole (DHI) 		Chemical degradation methods, HPLC, ESR spectroscopy	Photoprotective and antioxidant properties, unusual electrical conductivity	[21, 22]
	5,6-dihydroxyindole-2-carboxylic acid (DHICA) 		Chemical degradation methods, HPLC, ESR spectroscopy	Decreased visible light absorption and paramagnetic response relative to DHI-based melanins, markedly enhanced antioxidant properties	[21, 22]
Pheomelanin	5-S-cysteinyl-DOPAquinone 		Chemical degradation methods, HPLC,	Phototoxic (can lead to ROS production)	[21, 23, 24]
Pyomelanin	Benzoquinone acetic acid 		Fourier-transform infrared (FTIR), solid-state NMR (ssNMR), gel permeation/size exclusion chromatography (GPC/SEC)	Water-soluble, non-cytotoxic, radical scavenging activity, hyper thermostable	[25–27]
DHN-melanin	1,8-DHN 	–	EPR, solid-state NMR (ssNMR), UV–Vis and IR spectra	Redox and radical scavenging activities, antimicrobial activity	[28, 29]

from 10 to 14 kDa. This relatively low molecular weight allows it to cover the surface of cell walls, protecting fungi against external stress [35]. During the polymerization of pyomelanin, the $-CH_2COOH$ functional group remains unaltered, whereas the ortho position of the phenol undergoes an oxidative coupling reaction to form a quinone moiety. Because pyomelanin contains large populations of quinone molecules, it has exceptional electron transfer and reversible redox capacities [36]. Numerous functional groups, such as −OH, C═C, C═O, and COO−, have also been confirmed in pyomelanin [27].

DHN melanin is the most common type of allomelanin, which is derived from oxidization of 1,8-DHN subunits. In contrast to DOPA-melanin, DHN melanin precursors (acetyl CoA and malonyl CoA) are generated intracellularly, resulting in its typically close relationship with the cell wall. The presence of granular or fibrous DHN melanin in the cell wall primarily acts as a barrier to regulate the transport of cellular materials [37]. Furthermore, DHN melanin is a composite entity composed of loosely connected methylene and unsaturated moieties, where the aromatic carbons of indole are frequently substituted [33, 34]. This characteristic substitution pattern contributes to the heteropolymeric architecture of DHN melanin and resistance to all solvents [38]. However, the exact molecular interactions between DHN melanin and fungal cell walls have not yet been fully understood, nor is the process by which these monomers assemble into the final biopolymers.

Production of fungal melanin

Melanin-producing fungi found in natural environments

An ideal melanin-producing microorganism should have a rapid growth rate, exceptional industrial production

metrics (titer, rate, and yield), cost-effectiveness, and environmentally friendly characteristics. At present, several fungal species can generate melanin with noticeable diversity in terms of type and quantity, thereby providing a vast repertoire of natural resources for melanin biosynthesis.

For instance, *Auricularia auricula*produces a significant quantity of 2.97 g/L of melanin (including eumelanin and pheomelanin) through submerged fermentation, exhibiting remarkable resistance to high temperatures and light exposure [39]. After 168 h of incubation, halophilic black yeast *Hortaea werneckii* can achieve a yield of 5.60 g/L eumelanin [40]. Notably, pigment extraction from oyster mushrooms *Pleurotus cornucopiae* results in a mixture of eumelanin and pheomelanin, with a yield of 11 mg of pigment per gram of mushroom tissue [41]. Additionally, *Aspergillus carbonicus* can produce substantial melanin during 15–25 incubation days, achieving a maximum yield of 20.76 g/L [42]. Importantly, the *Basidiomycete Armillaria cepistipes* produces 27.98 g/L of eumelanin, the highest yield reported to date; however, the fermentation period is lengthy, lasting 161 days [43]. Eumelanin and pyomelanin have been utilized across various fields due to their exceptional antioxidant and electrical properties, while DHN-melanin has limited applications because of its ambiguous structure and challenging separability. Despite the significant challenge of prolonged fermentation time in industrial-scale fungal melanin production, improvements can be achieved through various strategies, including optimization of fermentation conditions, strain enhancement, and genetic engineering techniques, which will be discussed in the following section.

Fermentation

DOPA melanin is prioritized for industrial production due to its advantageous properties and its endogenous synthesis in the cell, even though its strong adhesion to the cell wall makes isolation and purification challenging [44]. In addition to selecting high-yielding melanin-producing classic strains, several factors, including nutritional components (precursors, fermentation substrates, carbon and nitrogen sources, metal ions, inducers), physical parameters (temperature, humidity, pH, light intensity, ventilation), and cultivation duration should be considered during fermentation process [9, 44, 45].

The optimal nutrition substrates for fermentation are based on strain-specific variations. For instance, glucose serves as the optimal carbon source, while peptone is the preferred nitrogen source for achieving a significantly high yield of melanin produced by *Yarrowia lipolytica* [46]. Conversely, *A. auricula* has a preference for lactose and yeast extract as substrates for melanin synthesis [47]. A range of agricultural and industrial residues, including wheat bran [48], corn cob powder [49], soybean meal [50], grape waste [51], rice bran [40], and other sources, have been regarded as potential nutrient substrates for reducing the costs of melanin production. *A. auricula* generates a yield of 0.9–1.1 g/L of melanin in a fermentation medium where wheat bran juice is the major component [52].

Fungal fermentation mainly requires a neutral or slightly acidic environment and the optimal humidity level is approximately 70% [53]. A total dark environment and proper ventilation can improve dry weight and melanin production in fungi [54, 55]. In practical applications, the response surface methodology (RSM) can be employed to develop a statistical model that identifies the optimal combination of factors for maximizing melanin production [56]. Notably, the melanin production of *A. auricula* has been significantly enhanced through meticulous optimization of fermentation conditions, achieving an impressive increase from 306.52 to 1008.08 mg/L. This profound achievement corresponds to a significant 3.29-fold increment in yield, exemplifying the efficacy of fine-tuning fermentation parameters in augmenting fungal melanin production [56]. Nonetheless, these fermentation parameters are often empirical and can be significantly influenced by the unstable quality of raw materials.

Induction mechanisms for fungal melanin synthesis

The induction of melanin synthesis involves the introduction of various precursors for the production of L-tyrosine; for instance, glucose serves as a cost-effective substrate for industrial melanin production [57]. The addition of L-tyrosine has significantly increased the melanin production of *A. auricula* and the yeast *Y. lipolytica W29* [39, 58]. Copper ions are important enzymatic cofactors in pigment production and are considered as a basic component of the fermentation medium. The gradual addition of $CuSO_4$ in the concentration range of 0.01–0.2 g/L improves tyrosinase activity and once the limit is exceeded, excessive metal ions can cause melanin precipitation [39, 48]. Furthermore, the introduction of lactic acid into the tyrosine medium could accelerate the enzymatic browning process in fungi, resulting in a consequential amplification of melanin biosynthesis [46]. In addition, salicylic acid is known to induce melanin synthesis in *Auricularia auricula-judae*, however, the underlying mechanism remains elusive [59]. In contrast, studies have demonstrated that bicyclic phenolic compounds positively influence melanogenesis in *Paecilomyces variotii* and *Aspergillus carbonarius* [42]. To scale up melanin production effectively, it is essential to select economically viable inducers that can manage production costs at an industrial scale.

Extraction and purification

Melanin is vulnerable to alkaline or oxidative stress; therefore, alkaline extraction followed by acid precipitation is commonly employed for structural identification [60]. Cavitation-based extraction (CE) is a time-saving alternative to the conventional extraction methods, leveraging the phenomenon of cavitation. This technique relies on the collapse of cavities formed in the liquid to facilitate the release of cellular material [16]. Moreover, advancements in this strategy have led to the incorporation of ultrasonic, microwave, and hydrodynamic methods to enhance the extraction process [61]. Furthermore, the enzymatic extraction method possesses advantages, such as high efficiency, mild conditions, and flexibility. Specific enzymes are utilized to degrade cell wall components, allowing melanin to be liberated from its complexes. Using a combined enzymatic approach, the extraction efficiency for melanin from the fruiting body of *Inonotus hispidus* reaches 74.6% [62].

Melanotic yeasts exhibit slow growth rates and are mainly unable to withstand hostile microorganisms, hindering their isolation. Quan et al. [63] successfully enriched black yeast with aromatic hydrocarbon, facilitating artificial cultivation. Furthermore, providing optimal growth conditions (e.g., suitable pH, moisture, temperature, copper content, cultivation time, and nutrition substance) for melanogenic strains can maximize pigment production [64, 65]. After a scalable production of the natural melanin, numerous purification operations should be performed, involving centrifugation, acid precipitation, re-centrifugation, and repeatedly distilled water rinsing, eventually resulting in achieving pure melanin particles [66]. However, there is no single standard method for melanin extraction and purification that can be universally applied. Strategies must target the specific host strains and types of melanin involved, and each step necessitates preliminary exploration. Therefore, future research should concentrate on unraveling the biosynthetic pathways, regulatory networks, and transport mechanisms associated with melanin synthesis in fungi, providing a critical theoretical foundation for the scalable production of such imperative biomaterials.

Biosynthesis of fungal melanin

Melanin is abundantly detected in fungi, even in white colonies of *Candida albicans* [67]. Melanin production is not merely a process of producing a single type of melanin; rather, it is a pigment-deepening process, involving various precursors [68]. *Ascomycetes* and some imperfect fungi employ the polyketide pathway, utilizing the endogenous substrate 1,8-dihydroxy naphthalene (DHN) as a precursor. In contrast, *A. niger* and *basidiomycetes* utilize an alternative pathway that employs L-3,4-dihydroxyphenylalanine (L-DOPA) as a precursor, resembling the biosynthesis of melanin in mammals [69].

As illustrated in Fig. 1, two pathways responsible for fungal melanin synthesis have been identified: the DHN pathway and the L-DOPA pathway. Specific enzyme blockers are used to prevent melanin synthesis, thereby unraveling the fungal melanin biosynthetic pathway [70]. Table 2 summarizes genes involved in melanin synthesis in various fungal species. Therefore, this review classified fungal melanin into four categories: DOPA-melanin (including eumelanin and

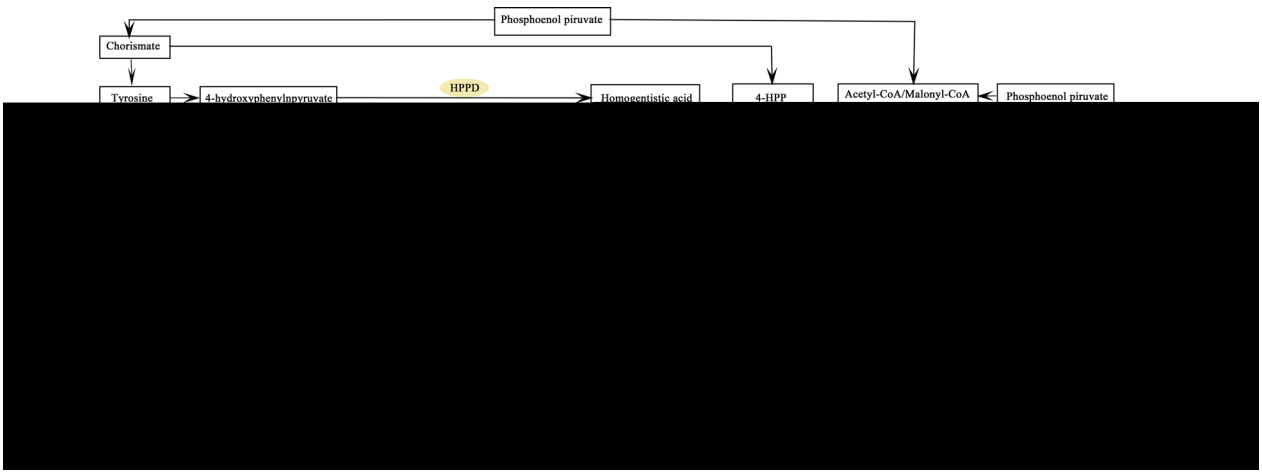


Fig. 1 Schematic representation of fungal melanin synthesis. *DHI* 5,6-dihydroxyindole, *DHICA* 5,6-dihydroxyindole-2-carboxylic acid, *TRP1* tyrosinase-related-protein-1, *TRP2* tyrosinase-related-protein2, *PKS* polyketide synthase, *YWA1* yellow pigment intermediate of WA polyketide synthase, *PAP* P-aminophenol, *GHB* 4-glutaminylhydroxybenzene, *γ-GT* γ-glutamyl transpeptidase, *HPPD* 4-hydroxyphenylpyruvate dioxygenase [87], *T4HN reductase* 1,3,6,8-tetrahydroxynaphthalene reductase [91], *SCD* scytalone dehydratase [84], *TPS1* trehalose-6-phosphate [T6P] synthase [92], *TPS2* T6P phosphatase [92]

Table 2 Melanin biosynthetic genes in different fungal species

Enzymes	Conidial/Mycelial color of mutant strains	Genes in different species	References
Polyketide Synthase B	White	*Aspergillus fumigatus*, *Aspergillus carbonarius*, *Magnaporthe grisea*, *Magnaporthe oryzae*, *Pyricularia oryzae*, *Penicillium chrysogenum*, *Metarhizium acridum* (*Alb1*), *Aspergillus nidulans*, *Aspergillus flavus*, *Aspergillus oryzae* (*wA*), *Aspergillus niger* (*FwnA*), *Alternaria alternata* (*PksA*, *Alm*), *Wangiella dermatitidis*, *Colletotrichum lagenarium*, *Metarhizium robertsii*, *Metarhizium rileyi* (*Pks1*), *Botrytis cinerea* (*Pks12/13*), *Pestalotiopsis fici* (*MaE*)	[74–82]
A/B Hydrolase	Yellowish Green	*Aspergillus fumigatus*, *Penicillium chrysogenum*, *Penicillium marneffei*, *Verticillium dahliae* (*Ayg1*), *Aspergillus niger*, *Exophiala dermatitidis* (*Olva/Ayg1*), *Alternaria alternata* (*AygA/B*), *Wangiella dermatitidis*, *Botrytis cinerea* (*Yg1p*)	[72, 77, 81, 82]
T4HN Reductase	Reddish Pink	*Aspergillus fumigatus*, *Penicillium chrysogenum*, *Penicillium marneffei* (*Arp2*), *Alternaria alternata* (*Brm3*), *Verticillium dahliae* (*Th4r*), *Metarhizium acridum* (*Thr*)	[72, 76, 77, 81–83]
Scytalone Dehydratase	Reddish Pink	*Aspergillus fumigatus*, *Penicillium chrysogenum*, *Penicillium marneffei* (*Arp1*), *Alternaria alternata*, *Botrytis cinerea*, *Bipolaris oryzae*, *Colletotrichum lagenarium* (*Brm1, Scd*), *Pyricularia grisea*, *Verticillium dahliae* (*Sdh*)	[72, 77, 81, 82, 84]
Laccase	Brown	*Aspergillus fumigatus*, *Penicillium chrysogenum*, *Penicillium marneffei*, *Metarhizium acridum* (*Abr1/2*), *Aspergillus nidulans*, *A. oryzae* (*yA*), *Aspergillus niger* (*BrnA*), *Aspergillus flavus*(*olgA/gldA*), *Alternaria alternata* (*IccA-G*), *Cryptococcus neoformans*, *Verticillium dahliae*, *Metarhizium acridum*, *Metarhizium rileyi* (*Lac1/2*)	[72, 76, 77, 81, 82, 85]
Dioxygenase	White	*Aspergillus fumigatus* (*HppD/A*)	[86]
Cytosolic Protein	Yellowish Green	*Aspergillus fumigatus* (*HmgX*)	[87]
1,3,8-Reductase	–	*Alternaria alternata* (*Brm2*), *Botrytis cinerea* (*brn1/2*), *Verticillium dahlia* (*Th3r*)	[79, 82]
Tyrosinase	Fluorescent Yellow	*Aspergillus terreus* (*TyrP*), *Metarhizium acridum* (*Tyr1/2*)	[76, 77]

pheomelanin), DHN melanin, pyomelanin, and others [39, 71]. However, most fungi possess multiple melanin biosynthetic pathways, while *Cryptococcus neoformans* exclusively synthesizes melanin through the L-DOPA pathway. This unique characteristic makes it a notable model organism for studying melanogenesis in fungi [72, 73].

DOPA-melanin

Eumelanin is a sulfur-free pigment, whereas pheomelanin contains sulfur due to cysteine integration. Both are preferentially synthesized in fungi via the L-DOPA pathway and contribute to virulence in association with infection and phagocytosis. In the L-DOPA pathway, tyrosine or phenylalanine is used as the substrate to form DOPA, which can subsequently be converted into DOPA quinone (DAQ) under the catalytic action of tyrosinase. This is an important rate-limiting step that can regulate the melanin synthesis process. DAQ then undergoes a series of oxidative polymerization reactions to separately generate eumelanin and pheomelanin (Fig. 1) [88, 89]. Tyrosinase (EC 1.14.18.1) or laccase (EC 1.10.3.2) are the major enzymes in L-DOPA melanin production. In contrast to *Aspergillus fumigatus* consuming tyrosine to synthesize DOPA melanin [90], *Cryptococcus neoformans* directly selects DOPA as the substrate and subsequently oxidizes DOPA to melanin only by laccases (*lac1* and *lac2*) [73].

DHN-melanin

Allomelanin is a complex heterogeneous pigment composed of various dark pigments, typically derived from nitrogen-free precursors, including catechol, HPQ (1,4,6,7,9,12-hexahydroxyperylene-3,10-quinone), and DHN [93, 94]. The most prevalent form of allomelanin, DHN melanin, is an endogenous pigment involved in the formation of the rodlet layer with RodA hydrophobins. This serves as a strategy to evade host immune responses by masking pathogen-associated molecular patterns (PAMPs) [95]. The biosynthetic steps include acetyl-CoA or malonyl-CoA, acting as the substrate, and polyketide synthase (PKS), catalyzing the formation of intermediate-1,3,6,8-tetrahydroxynaphthalene (T4HN) [91]. After T4HN formation, scytalone is produced under T4HN reductase before undergoing dehydration to form 1,3,8-THN. After a series of reduction and dehydration reactions, DHN-melanin is eventually formed by laccase-catalyzed polymerization (Fig. 1) [12].

Of note, the gene cluster responsible for DHN melanin synthesis in *A. fumigatus* contains six genes (*pksP/alb1, ayg1, arp1, arp2, abr1*, and *abr2*), with the first four genes encoding cytoplasmic enzymes, while the last two genes encode typical secretory proteins. However, some differences exist in the DHN melanin synthesis pathway in different fungi. For example, T4HN may not be directly catalyzed by polyketide synthase in some fungi. Polyketide synthases (encoded by *pksP/alb1*) from *A. fumigatus*

and *Exophiala dermatitidis* produce heptapeptide naphthopyranone YWA1, followed by deacetylation of the chain-shortening enzyme Ayg1p to form T4HN. Additionally, *Botrytis cinerea* has a non-linear DHN melanin synthesis pathway containing two polyketide synthases, i.e., *BcPKS12* and *BcPKS13*, involved in the melanin synthesis of sclerotia and conidia, respectively [79, 96].

Pyomelanin

Pyomelanin is a water-soluble pigment that is synthesized extracellularly. It can bind to the surface of hyphae, thereby playing a pivotal role in both cell wall integrity and virulence [97]. The biosynthetic pathway of pyomelanin is mediated by the DOPA and DHN pathways. In *A. fumigatus*, pyomelanin is synthesized from tyrosine through the tyrosine degradation pathway, which produces homogentisic acid (HGA), a crucial intermediate. When HGA accumulates to a certain level, it can spontaneously oxidize to form benzoquinone acetate and polymerize to produce pyomelanin. There is a gene cluster that includes six genes (*hppD*, *hmgX*, *hmgA*, *fahA*, *maiA*, and *hmgR*) responsible for its synthesis [86]. Disrupting the expression of *hppD* in *A. fumigatus* can result in a light color phenotype, attributed to the absence of HGA. A cofactor of the hppD enzyme, hmgX, influences pyomelanin production in *A. fumigatus* [87].

Others

Aside from DOPA/DHN-melanin, certain fungi contain a variety of other specific pigments. In *Agaricus bisporus*, for example, γ-glutamine-3,4-dihydroxybenzene (GDHB) or catechol can be used as a phenol precursor to form GHB-melanin or catechol-melanin via a series of oxidative polymerization reactions, which contribute to their browning susceptibility [98]. Additionally, *Aspergillus terreus* can produce a type of cinnamon-brown Asp-melanin pigment during conidiation. The synthesis begins with the condensation of two molecules of p-hydroxyphenylpyruvate by a non-ribosomal peptide synthetase-like (NRPS-like) enzyme (MelA) to form aspulvinone E, which is then converted into Asp-melanin by tyrosinase (*TyrP*) [99]. In addition to these pigments, PAP-melanin synthesis is also notable. The biosynthetic pathway for PAP-melanin involves the initial conversion of phenylalanine to p-hydroxyphenylpyruvate (HPP) by the enzyme phenylalanine ammonia-lyase (PAL). HPP is then further processed by enzymes such as HPPD, which catalyzes the formation of 4-hydroxybenzoic acid, and subsequent reactions lead to the polymerization of these intermediates to produce PAP-melanin. This pathway is depicted in Fig. 1, illustrating the key enzymes and steps involved in PAP-melanin biosynthesis.

Transportation and localization

Melanin can be classified into three types based on their localization: melanin anchored in the cell wall (DOPA melanin), DHN-melanin, which is typically associated with the cell wall but can also be found in the intracellular space, and free extracellular water-soluble pigment (pyomelanin) [95]. This classification highlights the varying distribution of these melanins in fungal cells and their environments. Melanocytes are specialized cells responsible for the synthesis and storage of melanin. They contain unique lysosome-related organelles (LROs) known as "melanosomes," serving as the site for melanogenesis. Eumelanosomes are ellipsoidal vesicles characterized by a fibrous matrix, while pheomelanosomes consist of several small, globular matrices without a fixed shape. The development of melanosomes in mammals occurs in four stages. In stages I and II, ribosomes transport dynamic tyrosinase enzymes to the melanocytes, facilitating the formation of interlamellar fibers in the melanosomes. During stage III, the melanosome begins synthesizing melanin, which is deposited along the fibers, gradually darkening the vesicles. Finally, in stage IV, the sparse protein wall is filled with the participation of various active enzymes [29, 100].

After producing melanin in melanosomes, the biopolymers can be transported to the cell wall through the interaction between melanocytes and keratinocytes. Melanocytes can transfer melanin to the keratinocyte via two transit models, including the shedding-phagocytosis model and the endocytosis model [101]. In the former one, melanocytes release melanin vesicles rich in melanin, which are thereafter internalized by keratinocytes via phagocytosis. In contrast, melanocytes fuse with the cell membrane and directly release melanin granules into the extracellular matrix in the latter model; eventually, keratinocytes absorb melanin via endocytosis [102].

Numerous fungal species, including *C. neoformans*, *C. albicans*, *Cladosporium carrionii, Fonsecaea pedrosoi,* and *A. terreus* [77, 100, 103], contain vesicles loaded with laccase, which are analogous to mammalian melanosomes. The mechanisms underlying the synthesis and transport of these vesicles appear to be similar to those in mammals [100]. Melanin granules can be released from extracellular vesicles and subsequently deposited on the cell wall, forming concentric layers that provide protection against external stressors. Chitin, an essential component of the fungal cell wall, acts as a critical scaffold for the binding of melanin to the cell wall matrix [104–106]. Disruption of the chitin synthase gene *CHS3P* inhibits the anchoring of melanin granules in the cell wall [104]. Furthermore, enzymes involved in melanin synthesis are specifically compartmentalized in the cell. Early biosynthetic enzymes are often stored in melanosomes, whereas

late biosynthetic enzymes are concentrated in the cell wall. This evidence supports the above-mentioned melanin melanogenesis model [107].

Regulation of melanin synthesis

Fungal melanin regulation is complex, and it is significantly influenced by nutritional and environmental factors that govern melanin synthesis. Melanin production depends highly on the activities of rate-limiting enzymes, such as tyrosinase, polyketide synthase (PKS), and laccase. Therefore, the expression levels of genes encoding the enzymes can significantly influence the melanin synthesis process, which can be mediated by related upstream pathways and transcriptional factors (TFs). Lee et al. found four melanin-regulating core TFs (*Bzp4*, *Usv101*, *Mbs1,* and *Hob1*) and potential upstream kinases, providing noticeable insights into the core melanin regulatory network in *C. neoformans* and other fungal pathogens [108]. Supplementary Table S1 presents a compilation of melanin regulatory components from a variety of organisms. In this section, *C. neoformans* has been adopted as the model organism for summarizing a range of regulatory elements involved in melanin biosynthesis. A visual representation of these factors is illustrated in Fig. 2, complementing the diagram proposed by Lee et al. [108], thereby enhancing the understanding of the fungal melanin regulation process.

Figure 2 presents various molecules and proteins, with their functions detailed as follows: Chs3 acts as Chitin Synthase (CHS) [105], Msb2 as a putative osmosensor [109], Opy2 as a transmembrane-anchor protein [109], Sln1 as a histidine kinase [144], and Sho1 as an osmoreceptor [110]. Ypd1 functions as a phosphotransfer protein, Cdc42 as a small G protein, Ssk1 as a response regulator, Ste20 as a PKA kinase, Ssk2/22 as MAPKKK, Ste50 as a bridging protein, Ste11 as MAPKKK, and Pbs2 and Hog1 as MAPKK [111]. Mob2, Hym1, and Sog are associated proteins, while Cbk1 and Kic1 are Ser/Thr protein kinases [112]. Ace2 and Ssd1 function as a transcription factor (TF) and an mRNA-binding protein, respectively [111]. Ccc2 and Atx1 serve as a copper transporter and copper chaperone, respectively [73], while Ctr1 is a high-affinity copper transporter [73]. Gsk3 and Pro1 act as a core kinase and TF, respectively [112]. Core melanin-regulating TFs include Bzp4, Usv101, Mbs1, and Hob1 [108], with Pkh202 as a TF [112]. Met3, Mps1, and Mec1 are kinases [108], Lac1 is a laccase type [73], and Vad1 and Not1 function as an RNA helicase and global transcription repressor, respectively [113]. Vps15, Vps30, Vps34, and Cig1 are involved in vacuolar protein sorting,

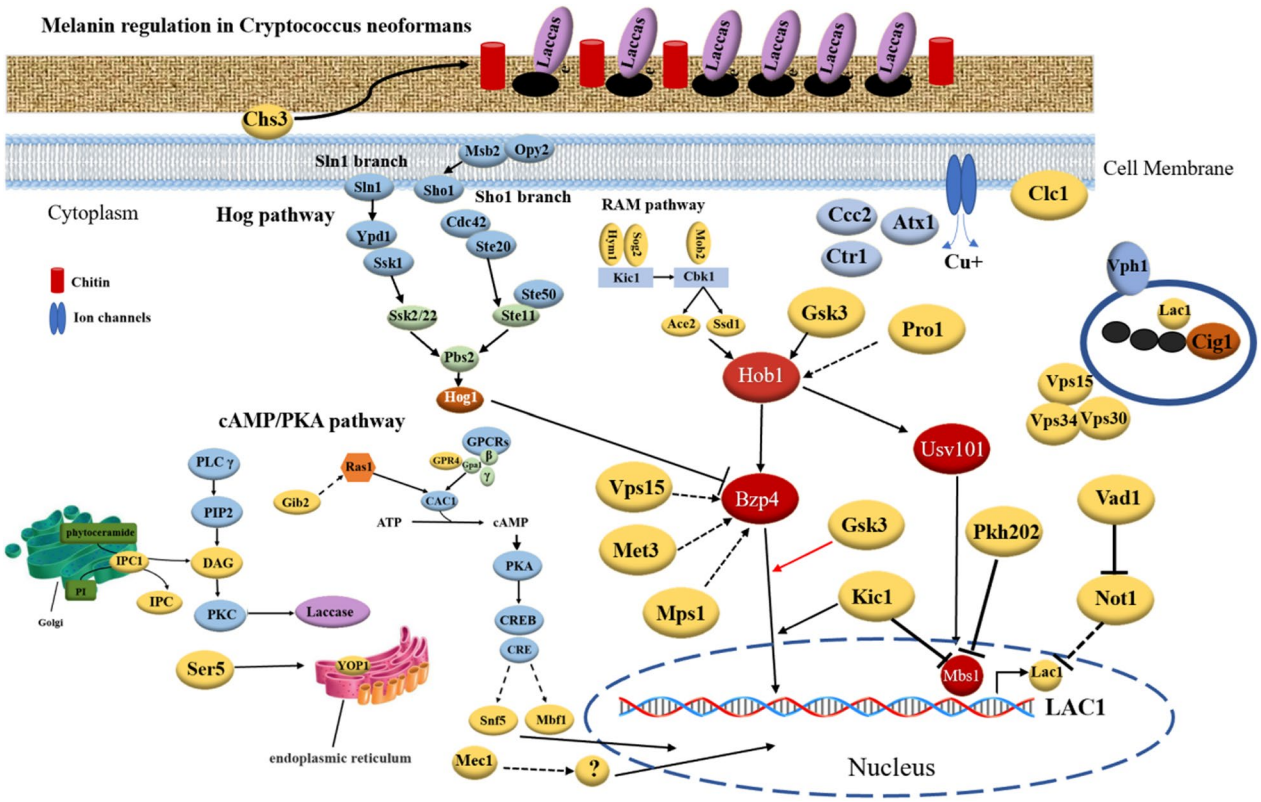


Fig. 2 Regulatory network of melanin synthesis in *Cryptococcus neoformans*

functioning as a Ser/Thr kinase, vesicle-associated membrane protein, phosphatidylinositol 3-kinase, and mannoprotein, respectively [108]. Vph1 is a component of the vesicular proton pump [114], Clc1 is a voltage-gated chloride channel [115], Gpr4 is a cAMP receptor [116], Gpa1 is a G-protein alpha subunit [117], CAC1 is an adenylyl cyclase [118], Gib2 is a Gβ-like/RACK1 protein [116], and Ras1 is a GTPase protein [118]. PKA represents Protein Kinase A, CreB is a cAMP response element-binding protein, and Cre is a cAMP response element [116]. Snf5 is a chromatin-remodeling protein, and Mbf1 is a transcriptional coactivator [73]. Additionally, PLC denotes Phospholipase C, PIP2 denotes Phosphatidylinositol (4,5) Bisphosphate, DAG denotes Diacylglycerol, PKC denotes Protein Kinase C, PI denotes Phosphatidylinositol, IPC denotes Inositol Phosphorylceramide, and IPC1 denotes Inositol Phosphorylceramide Synthase 1 [119]. Finally, Ser5 represents Serine protease 5 [120], and YOP1 is an endoplasmic reticulum curvature-stabilizing protein [121].

Environmental factors and related signaling pathways

Fungal melanin production requires the stimulation of various environmental signals (i.e., nutrient starvation), the presence of multivalent cations, and thermal stress, all depending on signal transduction pathways [121]. Several signal sensors (*Opy2-Msb2* complex, *Wsc1*, *Wsc3*, and *MidA*) in the cell wall detect external adverse stress, providing a specific signal-receptor regulation mechanism to activate melanin production [109, 122]. Furthermore, stress sensors, such as Bck1, Mkk2, and MpkA in the cell wall integrity (CWI) signaling pathway can activate pyomelanin biosynthesis in response to external stress [123].

In addition to cell surface receptors, the complete signal transduction process requires the involvement of classical signaling pathways. Several studies have demonstrated that the cyclic AMP/protein kinase A (cAMP/PKA) and high osmolarity glycerol (HOG) response pathways regulate melanin production. The stress-responsive HOG pathway comprises two major signaling modules, the MAPK module and the two-component system-like phosphorelay system, both of which suppress melanin synthesis in *C. neoformans* [124]. As illustrated in Fig. 2, there are two upstream signaling branches (*Sln* and *Sho1* branch) where different MAPKKKs (*Ste11* and *Ssk2/22*) converge a common MAPKK (*Pbs2*), transmitting signals to another MAPKK (*Hog1*). It was previously demonstrated that melanin biosynthesis is significantly repressed when the phosphorylation level of *Hog1* remains high [125, 126]. Furthermore, in response to nutritional and host signals, the cAMP/PKA pathway in fungal pathogens influences the expression of virulence determinants. Prior research documented that the elevated cAMP facilitates PKA's dissociation of the catalytic (Pka1 and Pka2) and regulatory (Pkr1) subunits, proving critical for downstream biosynthesis signaling [116]. Under melanin-inducing conditions, the transcriptional coactivator *Mbf1* and the downstream target *Snf5* act together to upregulate *LAC1* transcription in *C. neoformans* [73]. Deletion of the cAMP receptor *GPR4*, G protein α subunit *GPA1*, and a Gβ-like/RACK1 homolog *Gib2* could significantly reduce melanin synthesis in *C. neoformans* or *Cryptococcus gattii*, demonstrating the positive role of the cAMP/PKA signaling pathway in melanin production [116, 117]. Furthermore, two TFs (*MaMsn2* and *MaSom1*) in the cAMP/PKA signaling pathway have been found to affect melanin production in *Metarhizium acridum* (*M. acridum*) [127, 128].

Glucose can suppress *CnLAC1* expression in *C. neoformans*, indicating the potential involvement of the glycolysis pathway in melanin synthesis [129]. In *C. neoformans*, disruption of the glycolytic enzyme phosphoglucose isomerase (Pgi1) results in hypersensitivity to osmotic stress and a complete absence of melanin synthesis in mutants [130]. A similar effect was noted in *TPS1/2*, *T6P*, and *NTH1* mutants of *C. gattii* [92]. Furthermore, the growing interest among scientists in melanin phenotype research has revealed that the regulation of the morphogenesis (RAM) pathway influences melanin synthesis. The protein kinase *Kic1*, together with two related proteins, *Hym1* and S*og2*, phosphorylate *Cbk1* to activate the expression levels of the TF *Ace2* and the mRNA-binding protein *Ssd1*, emerging critical for cell separation and stress response regulation [108, 112]. This newly discovered connection provides a novel perspective on the regulation of *Lac1* expression in *C. neoformans*.

Rate-limiting enzymes in melanin synthesis

Tyrosinase (EC 1.14.18.1), laccase (EC 1.10.3.2), and PKS are the key rate-limiting enzymes in melanin synthesis, and their activity significantly affects melanin production in fungi. Tyrosinase is the primary enzyme involved in melanin synthesis. It catalyzes the two-step tyrosine oxidation process, leading to DAQ production. Tyrosinase contains a coupled binuclear copper center, in which two copper atoms (CuA and CuB) are coordinated by three histidine residues. This arrangement facilitates the catalysis of oxidation reactions of monophenols or o-diphenols to form quinones [131, 132]. Laccase, a "blue proteins" family member, contains four copper atoms bound to conserved histidine residues. The atoms are normally stored in the melanosomes and are critical to laccase's function. Two laccase-encoding genes, *LAC1* and *LAC2*, have been found in *C. neoformans*. The *LAC1*-encoded laccase was identified in the cell wall and reported to

play a dominant role in melanin synthesis, whereas *LAC2* was found in the cytoplasm, and its role remains elusive. The conversion of acetyl-CoA or malonyl-CoA to tetrahydroxynaphthalene (THN) in the DHN-melanin biosynthetic pathway is catalyzed by PKS [133]. Fungi frequently possess multiple PKS gene clusters as a result of gene duplication and horizontal gene transfer. In *Metarhizium*, two PKS gene clusters (*Pks1* and *Pks2*) exhibit distinct expression patterns. While *Pks1* is highly expressed during conidiation, *Pks2* exhibits the elevated activity during infection, demonstrating the functional division of the two gene clusters [134].

As previously described, both tyrosinase and laccase are copper-dependent enzymes, and the importance of copper in DHN and the L-DOPA melanin synthesis pathway cannot be overlooked [94]. For instance, *Ctr1,* the mutant of the copper transporter *Ccc2*, and the copper chaperone *Atx1* have been identified in melanin defects in *C. neoformans* [73]. Additionally, under copper-limiting conditions, the P-type Cu-transporter *CtpA* is required for melanization to transport copper from the cytosol to endosomes in *A. fumigatus* [107]. Furthermore, due to its unique localization, a group of genes associated with vesicle trafficking and metal homeostasis, including a vacuolar-type proton pump *Vph1*, a chloride ion channel *Clc1*, autophagic vesicle-associated proteins (*Vps30*, *Vps15*, and *Vps34)*, and the mannoprotein *Cig1* can influence laccase expression [55, 108].

Transcription factors (TFs) involved in melanin synthesis

TFs, key cellular components that control gene expression, can participate in eukaryotic transcriptional processes. Extensive research has revealed that TFs exert a significant regulatory effect on melanin biosynthesis in fungal systems. For instance, 27 novel TFs associated with capsule and melanin production have been identified in *C. neoformans* [135]. However, as studies mainly did not concentrate on the phenotype of melanin deficiency, integrating these TFs into established melanin synthesis pathways has been challenging. However, with the recent elucidation of the core melanin-regulating network in *C. neoformans* [108], further melanin regulation-TFs (MR-TFs) can be incorporated into an existing regulatory framework, providing new insights into melanin production in fungi.

The *Cmr1,* a melanin-specific transcriptional activator, is present in most melanin-producing fungi, specifically stimulating the expression of the *PKS* gene clusters. Consequently, in melanin-producing fungi, the *PKS* gene, *Arp2*, and the *Cmr1* gene exhibit a strong co-evolution [136]. Notably, deletion of the homologous genes in other species, such as *Amr1* in *A. brassicicola* [137], *VdCmr1* in *V. dahlia* [138], *Bcsmr1* in *B. cinerea* [139], *StMR1* in *S. turcica* [140], and *Pmr1* and *Pmr2* in *P. microspore* [141], significantly induces a melanogenesis deficiency phenotype. In addition to MR-TFs, some TFs also indirectly influence melanin synthesis regulation, although their precise underlying mechanism remains elusive. For instance, in *A. fumigatus*, *DevR* (the bHLH TF) and *RlmA* (the *MADS-box* TF) can recognize special sites in the promoter region of the *PksP* gene and bind to regulate melanin biosynthesis [142]. Additionally, deletion of *RsdA*, a regulatory factor involved in fungal secondary metabolism and development in *P. fici*, significantly enhances melanin production, resulting in a heavily pigmented hyphal phenotype, similar to that found in *PfmaF* and *PfmaH* TF mutants [143]. Furthermore, *Bcltf1/2* in *B. cinerea*, *Ncfl* in *N. crassa*, and *MoMyb7* in *M. oryzae* have also been identified as melanin regulatory factors [75, 144, 145].

Some TFs or proteases also regulate melanin synthesis in fungi with ambiguous melanin synthesis pathways. For instance, mutants of certain TFs (*MaNcp1*, *MaCreA*, *Mavib-1*, *MaMsn2*, and *MaSom1)* in *M. acridum* exhibited significantly lighter colony colors compared with the wild type [127, 128, 146–148]. Similarly, the alkylsulfatase gene *MaAts*, the dual-specificity cell division cycle 14 phosphatase gene *MaCdc14*, the conidial regulator *MaNsdD*, and the calcofluor white hypersensitive proteins *Macwh1* and *Macwh43* have exhibited to impact *M. acridum*'s resistance to environmental stress. These effects can highly be attributed to variations in melanin synthesis in the fungus [76, 149–151]. Although these studies did not illuminate the phenotype of melanin deficiency, they paved the basis for further research on melanin synthesis in *M. acridum*. Furthermore, additional melanin synthesis regulatory factors in other pathogenic fungal species have been elucidated in recent years. For instance, the appressorium membrane-specific protein *Pams1* in *M. oryzae* and the TF Mrap1 in *M. rileyi* have been found to regulate melanin density in the cell wall [152]. These studies have contributed to a deeper understanding of melanin synthesis in fungi.

Perspectives for efficient production of fungal melanin via metabolic engineering

In addition to the effective utilization of natural microorganisms [153], genetic engineering provides a practical approach to enhance melanin production by altering microbial genomes. These techniques enable the modification of microorganisms to yield soluble, pure, and high concentrations of melanin [13]. However, only microbial strains yielding 1 g/L can be considered for large-scale melanin production [77]. The typical metabolic engineering-based melanin production strategy includes overexpression of genes, encoding key melanin synthesis

enzymes, mutation of melanin-related inhibitor genes, and generation of recombinant organisms by introducing exogenous genes from other species with excellent melanogenic ability [154].

Bionic synthesis of eumelanin via tyrosinase is the closest approach to the in vivo biosynthesis process in fungi. Heterologous expression of the *P. sanguineus* tyrosinase in *A. niger*, where the gene is placed under a strong promoter for constitutive glyceraldehyde phosphate dehydrogenase (GAPDH), successfully induced the expression of a highly active tyrosinase that can be utilized downstream for melanin synthesis [155]. During pyomelanin synthesis, 4-hydroxyphenylpyruvate dioxygenase (HPPD) can oxidize 4-hydroxyphenylpyruvate (4-HPP) to homogentisate, followed by a series of oxidative polymerization reactions to form the end product. Overexpression of *F. kingsejongi* HPPD in *E. coli* results in a favorable melanin yield of 3.76 ± 0.30 g/L, achieving 62% of the yield by wild *F. kingsejongi* [156]. Besides directly modifying key enzymes in melanin synthesis, employing TFs to regulate target gene expression can be an effective method to enhance melanin production. For instance, overexpression of the TF *PfmaF* in *P. fici* [143] and the BMR_1 gene in *B. Oryzae* [157] resulted in a heavier pigment accumulation. Furthermore, knocking out the negative regulatory genes of melanin biosynthesis (*BcVeA* and *BcVelB*) in *B. cinerea*, increased melanin accumulation and enhanced sensitivity to oxidative stress in the mutants [153]. These findings convincingly demonstrate the viability and promise of genetic engineering technologies in fungal melanin production.

Furthermore, targeted modifications have significantly expanded the range of applications for engineered melanin [158]. For instance, modifications related to amino acids, carboxymethylation, D-glucosamine, and sulfation have significantly enhanced water solubility or self-assembly ability. Such deliberate modifications produce tailor-made melanin with unique photophysical and photochemical properties, opening up new directions for various fields [11, 39, 159, 160]. Therefore, gaining a more comprehensive understanding of melanin modification in fungi may provide a practical basis for developing engineered melanin with novel properties.

Applications of fungal melanin

The remarkable resilience of melanotic fungi in extreme environments positions fungal melanin as a highly valuable pigment across multiple fields, including industry, healthcare, environmental applications, and energy [13, 161]. The unique properties and scalable production of fungal melanin enable its broad functional applications. Significant advancements in fungal melanin use across various fields are outlined in the following sections of this article (Fig. 3).

Environment

The carboxyl, phenolic, hydroxyl, and amine functional groups in melanin make it an excellent candidate for bioremediation, which is extremely beneficial to environment management [161, 162]. The affinity of melanin to bind irons leads to its application in soil contamination remediation and wastewater purification. Melanotic fungal species have been utilized to immobilize heavy metals in polluted environments and degrade volatile organic compounds (VOCs) in the atmosphere. The presence of black fungus in cultures has been found to reduce VOC content by more than 96% after 48 h [163]. Additionally, electrospun membranes incorporating fungal melanin can effectively remove over 90% of heavy metals from contaminated water [164]. Similarly, *Aspergillus niger* can degrade 3 g/L sepia ink in saline industrial wastewater during 96 h to achieve wastewater purification [165]. Another notable property is the antimicrobial effect of fungal melanin, making it to be used as a biocontrol agent [166]. Pesticides containing melanin can be resistant to degradation, enabling them to retain better insecticidal activity [167].

Healthcare

The energy flow in melanized fungi ecologically paves the basis for the development of radiation-resistant materials and equipment using melanin. There is a ready market for spacecraft equipment, which protects astronauts from radiation exposure-induced side effects [168]. Besides, it is applicable in the development of oral melanin-based products and melanin nano-shells, assisting patients undergoing radiation therapy [7]. Melanin-based nanoparticles have been developed to help macrophages repolarize into an inflammatory phenotype via photothermal therapy (PTT) [169]. Meanwhile, biocompatible melanin-like particles can be utilized as an exceptional drug delivery carrier, not only supporting binding sites for medicine, but also providing superior imaging [170, 171]. Melanin-based nanoparticles have exhibited potential in tumor treatment through their role in PTT. This application leverages melanin's light-absorbing properties, where nanoparticles accumulate in tumor sites and convert light into heat, selectively targeting and destroying cancerous cells. Additionally, melanin's radiation-protective capabilities provide a potential direction for enhancing tumor treatment by protecting healthy cells while enabling radiation therapy.

The antioxidative role of fungal melanin in human health cannot be overstated. Experiments have demonstrated that mice fed with fungal-derived melanin exhibit

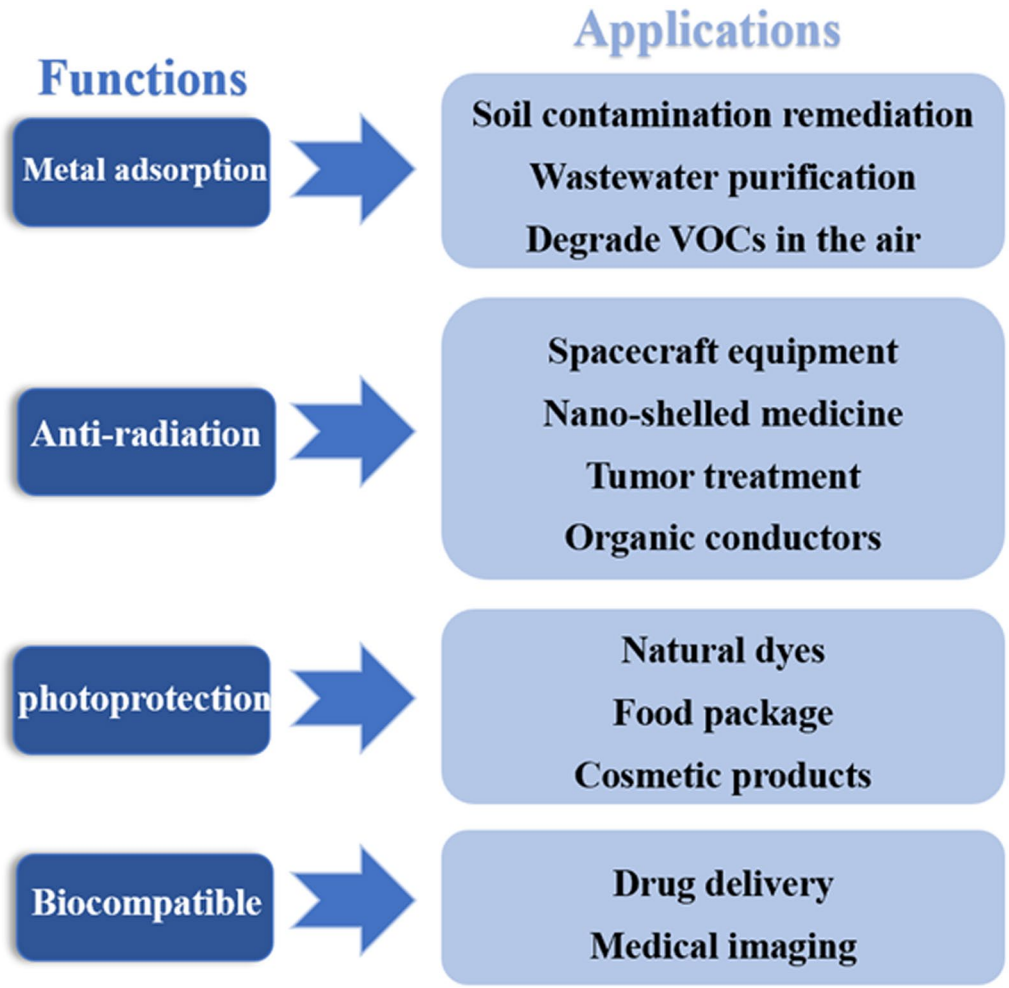


Fig. 3 A diagram illustrating the link between the function and application of fungal melanin

improved fat metabolism, enhanced insulin sensitivity, and reduced blood lipid and sugar levels [172]. Besides, melanin extracted from *A. auricula* can ensure faster oxidative stress response in mice suffering from alcoholic liver injury [173]. Melanin degradation also plays a pivotal role in human health, as it can influence the effectiveness of treatments for certain infections. For instance, inhibiting melanin synthesis in *Madurella mycetomatis* could improve its sensitivity to antifungal drugs, potentially enhancing therapeutic outcomes [174]. Numerous anti-melanogenic agents have been developed, including tricyclazole [175], coumaric acid [176], ellagic acid (EA) [177], paraquat (1,10-dimethyl-4,4-bipyridinium dichloride, PQ) [178], and dimethyl sulfoxide (DMSO) [88, 179], increasing their susceptibility to antifungal drugs.

Energy

Melanin pigments are regarded as excellent organic conductors owing to their unique physical and chemical properties [12, 180]. Recent studies have indicated that melanin can form an electron tunneling effect under radiation stimulation, enabling efficient electron conduction, providing a novel insight for the development of bio-semiconductor materials [181, 182]. Melanin-based electronic films have demonstrated electrical conductivities comparable with amorphous silicon, paving the basis for applications in chemi-sensors and bolometric photon detectors [183]. Coupled with its superior adversity tolerance, melanin is considered a soft biocompatible functional material. Organic electrodes comprising melanin have a superior capacity for charge storage [184]. Due to its remarkable stress tolerance and high energy capacity, fungal melanin also holds promising potential for energy-related applications.

Industry

In contrast to conventional synthetic dyes, fungal melanin possesses excellent benefits in terms of photoprotection

and antiradiation when applied in the textile industry, thereby protecting human skin from intense UV radiation [185]. Research has indicated that dyes made from fungal pigments have antimicrobial activities and excellent color fastness properties with no toxicity [186–189]. Fungal melanin has successfully entered the cosmetics and food industries, where it is utilized for coloring and packaging applications, positively promoting the sustainability of commercial products [9, 190, 191].

Conclusions

Fungi can synthesize various types of melanin, with properties that make fungal melanin one of the most valuable pigments across multiple fields. The remarkable structural diversity of these biopolymers contributes to their unique characteristics. Further elucidation of the detailed structures of different melanin polymers and linking these structures to diverse functions could enable the discovery and design of melanins with novel properties. Given the complex relationship between an organism's morphology and melanin's distinct properties, customized melanin could be developed through precise modulation of related enzymes. Such modifications could be performed within fungi or other organisms, or through direct engineering of fungal enzymes.

The natural production of melanin in fungal cell factories has significantly advanced green and sustainable manufacturing. Although optimizing fermentation conditions can substantially increase fungal melanin yields, further improvements in production efficiency are challenging. Fungal melanin synthesis is regulated by complex processes, involving environmental inducers, signaling pathways, rate-limiting enzymes, and TFs. Therefore, enhancing melanin production goes beyond optimizing fermentation processes and includes genetic regulation of target gene expression, broadening the industrial application potential of microbial systems.

Despite progress in optimizing fermentation conditions and bioengineering methods for melanin biosynthesis, substantial research remains to meet the demands of industrial-scale production, particularly due to the complex regulatory network governing fungal melanin synthesis. Future research will concentrate on understanding the molecular regulatory mechanisms to leverage genetic engineering in improving production efficiency and developing customized melanin with valuable material properties.

Abbreviations

TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
AFM	Atomic force microscopy
XPS	X-ray photoelectron spectroscopy
FT-IR	Fourier-transform infrared
EPR	Electron paramagnetic resonance
NMR	Solid-state nuclear magnetic resonance
HPLC	High-performance liquid chromatography
DHI	5,6-Dihydroxyindole
DHICA	5,6-Dihydroxyindole-2-carboxylic acid
VOCs	Volatile organic compounds
PPT	Photothermal therapy
EA	Ellagic acid
PQ	1,10-Dimethyl-4,4-bipyridinium dichloride
DMSO	Dimethyl sulfoxide
RSM	Response Surface Methodology
CE	Cavitation-based extraction
DHN	1,8-Dihydroxynaphthalene
L-DOPA	L-3,4-dihyroxyphenylalanine
DAQ	DOPA quinone
HPQ	1,4,6,7,9,12-Hexahydroxyperylene-3,10-quinone
PAMPs	Pathogen-associated molecular patterns
PKS	Polyketide synthase
T4HN	Intermediate-1,3,6,8-tetrahydroxynaphthalene
HGA	Homogentisic acid
GDHB	γ-Glutamine-3,4-dihydroxybenzene
NPRS-like	Non-ribosomal peptide synthetase-like
LRO	Lysosome-related organelles
cAMP/PKA	Cyclic AMP/protein kinase A
HOG	High osmolarity glycerol response
RAM	Regulation of the morphogenesis
THN	Tetrahydroxynaphthalene
MR TF	Melanin regulation transcription factors
HPPD	4-Hydroxyphenylpyruvate dioxygenase
4-HPP	4-Hydroxyphenylpyruvate
NQO	4-Nitroquinoline-1-oxide

Supplementary Information

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Supplementary material 1.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate

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Consent for publication

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Competing interests

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