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Regulation of melanin production in fungi

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Melanin is a dark macromolecule found in organisms ranging from animals to fungi and plants. In fungi, melanin is a secondary metabolite that is not essential per se for growth but does provide various benefits that facilitate adaptation to stressful conditions such as UV light, desiccation, oxygen radicals, and extreme temperatures. The biosynthetic pathways of most types of melanin are known and documented, but the regulation of those pathways is not well understood. In fungi, known pathways for melanin production include those directing the synthesis of 1,8-DHN melanin and L-DOPA melanin, as well as the tyrosine degradation pathway. Genetic studies have identified structural genes and enzymes that play a role in these different melanin biosynthesis pathways. Recent studies have focused on the roles of various transcription factors (TFs) and signaling circuits (e.g., cAMP/PKA and the HOG pathway) in regulating the expression of the biosynthetic pathways. The review will provide insights into what is known about these TFs and regulatory circuits in diverse fungi in an attempt to identify common themes.

KEYWORDS

fungal melanin, benefits of melanin, melanin biosynthesis, melanin biosynthetic pathways, transcription factors (TFs)

1 Introduction

Melanin is a dark, multifunctional pigment that is produced via the oxidative polymerization of phenolic and indolic compounds (Gomez and Nosanchuk, 2003; Solano, 2014; Suwannarach et al., 2019). The word melanin is derived from the Greek word "melanos," meaning "black" or "very dark" (Riley, 1997; Gessler et al., 2014). The term melanin was first used by the Swedish chemist Berzelius to name a dark pigment extracted from eye membranes in 1840 (Borovanský, 2011). Melanin does not refer to a single substance but a group of substances that have similar properties (Bell and Wheeler, 1986; Butler and Day, 1998; Langfelder et al., 2003). Because they originate from different starting precursors, melanin particles can be found in a range of shapes and sizes that include rods, platelets, and planar arrays (Glass et al., 2012; Song et al., 2023). Based on the chemical precursor and the biosynthetic pathway, melanin pigments are classified into five different types: eumelanin, pheomelanin, neuromelanin, allomelanin, and pyomelanin (Ambrico, 2016; Cao et al., 2021). These starting precursors condense and polymerize

into nanometer- to micron-size particles (Hong et al., 2012, 2018). Eumelanin, pheomelanin, and neuromelanin are found associated with animal tissues, whereas allomelanin and pyomelanin are found mostly in bacteria, fungi, and plants (Xie et al., 2019). Melanins are insoluble hydrophobic pigments that are negatively charged and have high molecular weight (Nosanchuk and Casadevall, 2003; Paolo et al., 2006; Solano, 2014). Another key feature of melanin is the presence of a stable free radical population (Sealy et al., 1982).

The coloration of melanin can vary, ranging from mainly dark brown to black, but in some instances, red or yellow coloration is also observed (Solano, 2014). Features that distinguish melanin from other secondary compounds such as carotenoids and polyketides include the following: i) melanin is extremely heat resistant and can withstand temperatures up to 600°C (Gallas et al., 2000), and ii) it is highly insoluble and resistant to strong acids, detergents, and reducing agents but is soluble in bases and phenols (Jacobson, 2000). Because of these features, the structure is quite hard to identify since classical methods using aqueous or organic fluids end up disrupting its organization (Nosanchuk et al., 2015). Like all naturally occurring pigments such as carotenoids, chlorophyll, and flavonoids, melanin contains conjugate moieties, such as aromatic rings, that allow electronic resonance and mediate energy transfer reactions (Cordero and Casadevall, 2017). Benefits and applied uses of melanin have been reviewed thoroughly (Cordero and Casadevall, 2017; Tran-Ly et al., 2020; Mattoon et al., 2021; Suthar et al., 2023). Although the biosynthetic pathways that produce melanin are reasonably well understood (Eisenman and Casadevall, 2011; Suthar et al., 2023; Qin and Xia, 2024), much less is known about the regulation that ensures the proper timing and location of production. Recent studies have implicated the PKA and HOG pathways as key signaling components of this regulation, while also identifying transcription factors that control the expression of the biosynthetic pathways. This review focuses on these recent advances and highlights remaining issues.

2 Fungal melanin

In general, fungal melanin is typically found in the outer regions of the cell wall though it can also be found clustered on the cell wall surface (Bayry et al., 2014). In some fungi, melanin acts as a structural component of spores, providing protection against various environmental stresses, increasing the survivability of the fungi (Xu et al., 2022). Melanin in many fungi is formed by a complex of differentially sized spherical particles that are approximately 200 nm in diameter and are known as melanin granules (Franzen et al., 2006; Kogej et al., 2007). These granules are composed of fungal melanosomes, which range from 30 to 120 nm in diameter. Melanin granules allow macromolecules to pass through the melanin, meaning that there are pores present in the melanin layers. Depending on the species, melanin tends to be stacked in layers with pores ranging between 1 and 4 nm in diameter to facilitate the passage of macromolecules (Eisenman et al., 2005; Casadevall et al., 2012). Since the cell structures of different fungi vary in their organization and materials, so does the stacking distance of the melanin layers; examples include 4.15 Å for *Exophiala dermatitidis* (also known as *Wangiella dermatitidis*), 4.45 Å for *Aspergillus niger*, and 4.39 Å for *Cryptococcus neoformans* (Nosanchuk et al., 2015). The indolic and/or phenolic monomers are ordered into planar arrangements of regularly interspaced stacked layers similar to graphite (Kim et al., 2016), and these layers can then cross-link into a more heterogeneous macromolecular configuration. This pattern of stacking is known as local-order-global-disorder and involves a combination of π-stacking, hydrogen, and ionic-bonded nanostructures with the melanin granules (Meredith and Sarna, 2006; Nosanchuk et al., 2015; Kim et al., 2016).

As the synthesis of melanin produces various highly reactive and toxic intermediates, fungal melanization occurs in specialized sphingolipid-enriched vesicles termed melanosomes, which are generally similar to mammalian melanosomes (Walker et al., 2010; Upadhyay et al., 2016). These vesicles contain laccase enzymes, leading to supramolecular buildup of melanin particles that are retained within the cell wall (Seiji et al., 1963; Camacho et al., 2019). The vesicles mediate the transport of intercellularly synthesized macromolecules to targeted sites on the cell surface where they can be captured by the cell wall (Eisenman et al., 2009). Studies have revealed that the melanin polymer is covalently bonded to cell wall chitin and is also found associated with other cellular moieties, including polysaccharides such as chitosan and plasma membrane-derived lipids (Zhong et al., 2008; Chatterjee et al., 2015). Evidence of this has also been provided whereby mutations affecting chitin synthesis genes in different fungal species, such as *E. dermatitidis*, *C. neoformans*, and *Candida albicans*, lead to a "leaky melanin" phenotype such that strains are able to synthesize melanin but the cell wall is unable to retain the melanin granules, which end up leaking into the extracellular space (Wang et al., 1999; Banks et al., 2005; Walton et al., 2005; Baker et al., 2007; Tsirilakis et al., 2012). Conversely, an increase in cell wall chitin or chitosan content reportedly increases melanin deposition (Banks et al., 2005; Baker et al., 2007; Tsirilakis et al., 2012). During the budding process of melanized yeasts, melanosomes in the cell wall are degraded or displaced, allowing daughter cells to emerge (Nosanchuk and Casadevall, 2003; Eisenman et al., 2005). Much of the work in understanding melanin structure and morphology has been performed on the so-called melanin "ghosts," which are macromolecular structures obtained after hot acid digestion of melanized cells (Eisenman et al., 2009; Chatterjee et al., 2015). Melanin ghosts are composed of smaller melanin granules that are arranged in concentric layers embedded within the fungal cell wall (Eisenman et al., 2005). Melanin produced by fungi varies depending on the species that produces it. Most ascomycetes produce 1,8-DHN melanin via the polyketide synthase pathway (Nosanchuk et al., 2015); another type of melanin, called L-DOPA melanin, is produced mainly by basidiomycetes (Nosanchuk et al., 2015). Species such as *Aspergillus fumigatus* and *A. niger* can produce multiple different melanin types, which presumably can act as a failsafe during stressful conditions if certain nutrient requirements are not met

(Pukkila-Worley et al., 2005). Some species such as *E. dermatitidis* have homologs of genes involved in 1,8-DHN melanin, L-DOPA melanin, and L-tyrosine degradation melanin pathways, but the exact mechanism or conditions that can trigger the production of L-DOPA or L-tyrosine melanin are not known (Paolo et al., 2006; Chen et al., 2014).

3 Fungal melanin biosynthesis

The main type of melanin produced by fungi, especially by ascomycetes, is 1,8-dihydroxynaphthalene (DHN) melanin via the polyketide synthase pathway (Nosanchuk and Casadevall, 2015). 1,8-DHN melanin is named after one of the pathway intermediates, 1,8-dihydroxynaphthalene, which was first identified in 1976 (Stipanovic and Bell, 1976). The second type of melanin, L-DOPA melanin, is named after one of the precursors, L-3,4-dihydroxyphenylalanine (Hamilton and Gomez, 2002). Besides the polymerization of 1,8-DHN, different species can also utilize other pigment precursors such as tyrosine, gamma-glutaminyl-4-hydroxybenzene (GHB), catechol, homogentisic acid, and scytalone (Bell et al., 1976; Weijin et al., 2013; Belozerskaya et al., 2016). The synthesis of eumelanin is catalyzed by phenoloxidases from L-DOPA substrates by fungal species such as *C. neoformans* (Langfelder et al., 2003; Nosanchuk and Casadevall, 2015; Tran-Ly et al., 2020). L-DOPA melanin is mainly synthesized by basidiomycetes, which occasionally also produce glutaminyl-3,4-dihydroxybenzene (GDHB) melanin (Henson et al., 1999; Selvakumar et al., 2008).

There are many fungal species that do not produce melanin under normal circumstances, but when supplemented with DOPA, they tend to produce L-DOPA melanin (Butler et al., 1989; Butler and Day, 1998). Despite the nature of the precursor, all fungal melanins tend to share similarities in functional groups and physiochemical properties (Fogarty and Tobin, 1996). During synthesis, several enzymes, such as tyrosinase, laccase, and catechol oxidase, carry out the rate-limiting initial oxidation of the starting phenolic precursors (Eisenman and Casadevall, 2012; D'Ischia et al., 2013; Solano, 2014), and the activity of these enzymes depends on the copper ions present at the catalytic site (Mauch et al., 2013; Upadhyay et al., 2013).

In fungi, the three different categories of melanin include 1,8-DHN melanin (allomelanin and pyomelanin), L-DOPA melanin (eumelanin and pheomelanin), and GHB melanin.

3.1 1,8-DHN melanin (allomelanin and pyomelanin)

The word "allo" refers to the Greek prefix meaning "heterogeneous" or "different" (Cao et al., 2021). The precursors of allomelanin can vary such that, depending on the precursor, allomelanins are referred to as 1,8-DHN melanin, HPQ melanin, or catechol melanin (Funa et al., 2005). The starting precursor for the synthesis of 1,8-DHN melanin, malonyl-CoA, was first identified by Fujii et al. (2000) in *Colletotrichum lagenarium*. Another precursor of 1,8-DHN melanin is acetyl-CoA, and both malonyl-CoA and acetyl-CoA are produced endogenously (Nosanchuk et al., 2015). These starting precursors are converted by polyketide synthase (PKS) to the first detectable intermediate 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN). 1,3,6,8-THN is reduced by hydroxynaphthalene reductase to produce scytalone (Alspaugh et al., 1997; Thompson et al., 2000). Scytalone is dehydrated enzymatically to 1,3,8-trihydroxynaphthalene (Alspaugh et al., 1998), which is then further reduced by a second reductase to vermelone (Basarab et al., 1999; Thompson et al., 2000). Vermelone is then further dehydrated by scytalone dehydratase to form the next intermediate 1,8-dihydroxynaphthalene (1,8-DHN). The pathway then involves a series of steps, including a dimerization of the 1,8-DHN molecules, followed by polymerization catalyzed by a laccase (Bloomfield and Alexander, 1967). 1,8-DHN proceeds through a C-C coupling reaction of the naphthalene rings, giving three 1,8-DHN dimers, which are then further oxidized to form a mixture of longer oligomers, which self-assemble to form the melanin structure (Figure 1) (Cecchini et al., 2017; Manini et al., 2018). The structure of the 1,8-DHN melanin polymer is not well known, but a study conducted by Beltran-Garcia et al. (2014) observed the presence of 50 1,8-DHN units in the polymer of melanin in the mycelium of *Mycosphaerella fijiensis*. The polyketide synthase responsible for the production of 1,8-DHN melanin generally possesses a similar structure across fungi, including a β-ketosynthase domain (β-KS), an acyl transferase domain (AT), and an acyl carrier domain (ACP). These are sometimes followed by a thiosterase domain (TE), which is responsible for detaching the polyketides from the enzyme (Watanabe et al., 2000; Fujii et al., 2001). 1,8-DHN melanin production can be inhibited by tricyclazole, pyroquilone, phthalide, and clobenthiazone (Selvakumar et al., 2008).

Like allomelanins, pyomelanins are derived from the oxidative polymerization of nitrogen-free precursors such as homogentisic acid (HGA) (Funa et al., 2005; Seo and Choi, 2020). Pyomelanin originates from the catabolism of either tyrosine or phenylalanine. The enzyme 4-hydroxyphenylpyruvic acid dioxygenase (HPPD) catalyzes the conversion of 4-hydroxyphenylpyruvate to HGA. Pyomelanin is generated through autooxidation to form benzoquinone acetic acid, which is then self-polymerized to form HGA and the pyomelanin polymer (Turick et al., 2010; Keller et al., 2011). Pyomelanin polymers tend to be smaller compared to other melanin pigments (Figure 1).

3.2 L-DOPA melanin (eumelanin and pheomelanin)

"Eu" is the Greek word for "good" or "well," and pheo means "dark" in ancient Greek (Cao et al., 2021). The main difference between eumelanin and pheomelanin is attributed to the potential of eumelanin (Figure 2a) to act as a photoprotector and the phototoxic nature of pheomelanin (Figure 2b) (Cao et al., 2021). Pheomelanins are also believed to contain benzothiazine subunits that are synthesized from L-DOPA and cysteine (Simon and Peles,

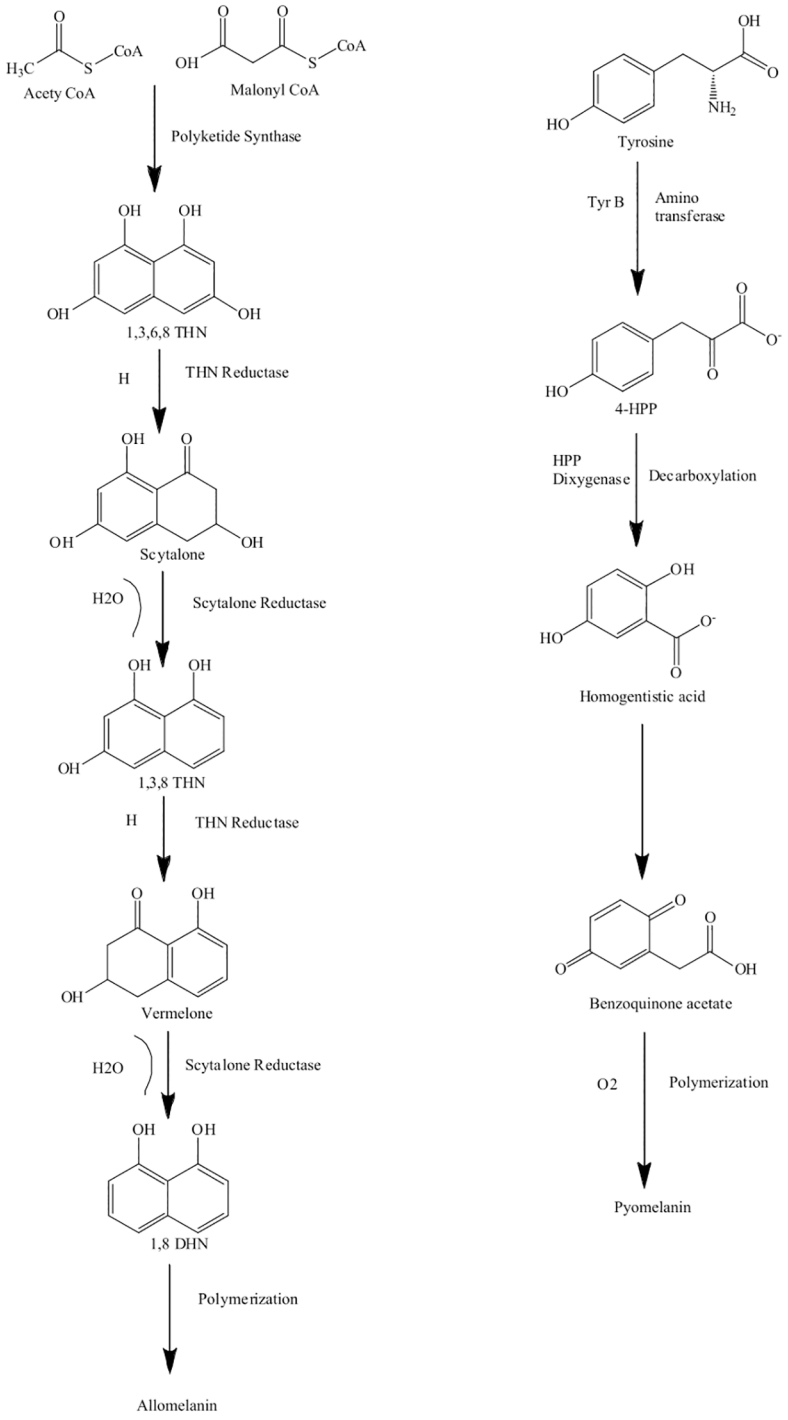

FIGURE 1
Biosynthesis pathway of allomelanin (1,8-DHN melanin) via the polyketide synthase (PKS) and pyomelanin derived from homogentisic acid. Pathways adapted from Suthar et al. (2023).

2010). Both eumelanin and pheomelanin are comprised of repeating units linked by carbon–carbon bonds (Costin and Hearing, 2007). Phenoloxidases for L-DOPA melanin can either be laccases or tyrosinases, both of which have copper ligands and require copper ions for activity. Both play different roles: laccases catalyze the one-step oxidation of dihydroxy phenols to quinones, and tyrosinases catalyze the two-step oxidation of tyrosine (Langfelder et al., 2003). In brief, the biosynthesis of eumelanin (Figure 3) begins with tyrosine, which is oxidized by oxygen, followed by tyrosinase that forms levodopa (L-DOPA) and then dopaquinone (Simon and Peles, 2010; Cao et al., 2021). During the L-DOPA melanin pathway, hydroxylation of L-tyrosine to

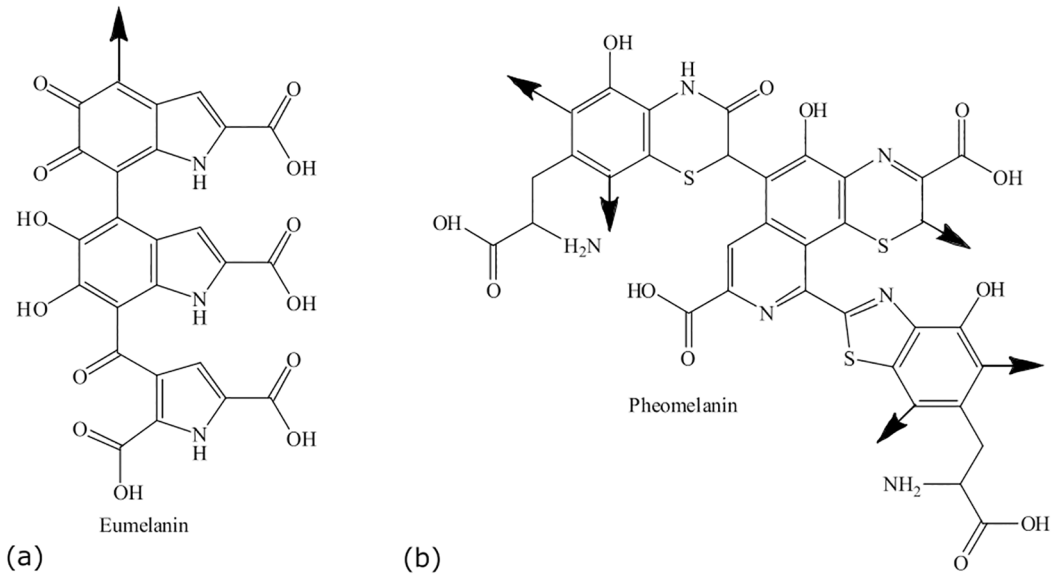

FIGURE 2
Structures of eumelanin **(a)** and pheomelanin **(b)**. Adapted from Sansinenea and Ortiz (2015).

dopaquinone or the oxidation of L-DOPA to dopaquinone is catalyzed by tyrosinase or laccase, respectively (Pomerantz and Warner, 1967). If there are no thiol groups present, dopaquinone forms leucodopachrome, which is then oxidized to dopachrome. Hydroxylation and decarboxylation then yield dihydroxyindoles, which are then further polymerized to form L-DOPA melanin (Ozeki et al., 1997a, 1997b; Butler and Day, 1998; Williamson, 1994). The synthesis of L-DOPA melanin has been shown to be inhibited by tropolone, kojic acid, and diethyldithiocarbamate (Salgado-Castillo et al., 2023).

3.3 GHB melanin

In mushrooms, such as *Agaricus bisporus*, another type of melanin referred to as GHB is present. γ-L-glutaminyl 4-hydroxybenzene (GHB) is found in the mycelium and the fruiting body of *A. bisporus*, whereas γ-L-glutaminyl-3,4_dydroxybenzene (GDHB) is found specifically in the reproductive hyphae (Stüssi and Rast, 1981). GHB melanin is also sometimes referred to as PAP melanin, where the initial substrate is *p*-aminophenol and the glutamyl groups are later removed before polymerization (Solano, 2014). GHB melanin is formed from either phenolic precursors or GHB via the action of a tyrosinase (Figure 4) (Weijin et al., 2013). Chorismate, which acts as the initial aromatic ring, is converted to *p*-aminophenol and conjugated with a glutamyl residue to form GHB. GHB can then be further oxidized to form glutaminyl-3,4-dihydroxybenzene (GDHB) or o-quinone (GBQ). The glutamyl residues are removed from the final pigment (Bisko et al., 2007).

4 Genes involved in melanin synthesis pathways

The identification of the genes involved in the production of 1,8-DHN melanin and L-DOPA melanin has been achieved using gene knockout strategies (Schumacher, 2016; Zhang et al., 2019; Nambu et al., 2021; Yang et al., 2022). The 1,8-DHN melanin synthesis gene cluster is conserved in many fungi (Schumacher, 2016; Ebert et al., 2018). The DHN melanin pathway in *A. fumigatus* is comprised of a cluster of six genes, namely, *abr1*, *abr2*, *ayg1*, *arp1*, *arp2*, and *pksP* (Perez-Cuesta et al., 2020). Pyomelanin synthesis is related to the L-tyrosine degradation pathway that includes a cluster of six genes: *hppD*, *hmgX*, *hmgA*, *fahA*, *maiA*, and *hmgR*. In *A. fumigatus*, the 1,8-DHN melanin biosynthetic gene cluster spans roughly 10 kb (Tsai et al., 1999). Genes encoded within this cluster are responsible for different steps of the 1,8-DHN melanin biosynthetic pathway. The PKS gene *alb1*, also referred to as *pksP*, participates in the β-keotacyl condensation of malonyl-CoA and acetyl-CoA to generate 2,5,6,8-tetrahydroxy-2-methyl-2,3-dihydro-4H-naphtho(2,3-b)pyran-4-one (YWA1) (Gao et al., 2022). During the second step, the *ayg1* gene hydrolyzes YWA1 to generate 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN). There are multiple reduction steps followed by aromatization/dehydration reactions that lead to oxidative polymerization (Perez-Cuesta et al., 2020). 1,3,6,8-THN is reduced to scytalone by the hydroxynaphthalene reductase gene *arp2* and the enzyme 1,3,6,8-reductase. The scytalone reductase gene *arp1* is responsible for the dehydration of scytalone to 1,3,8-trihydroxynaphthalene, which is followed by another reduction step by the hydroxynaphthalene reductase gene *arp2* that reduces 1,3,8-

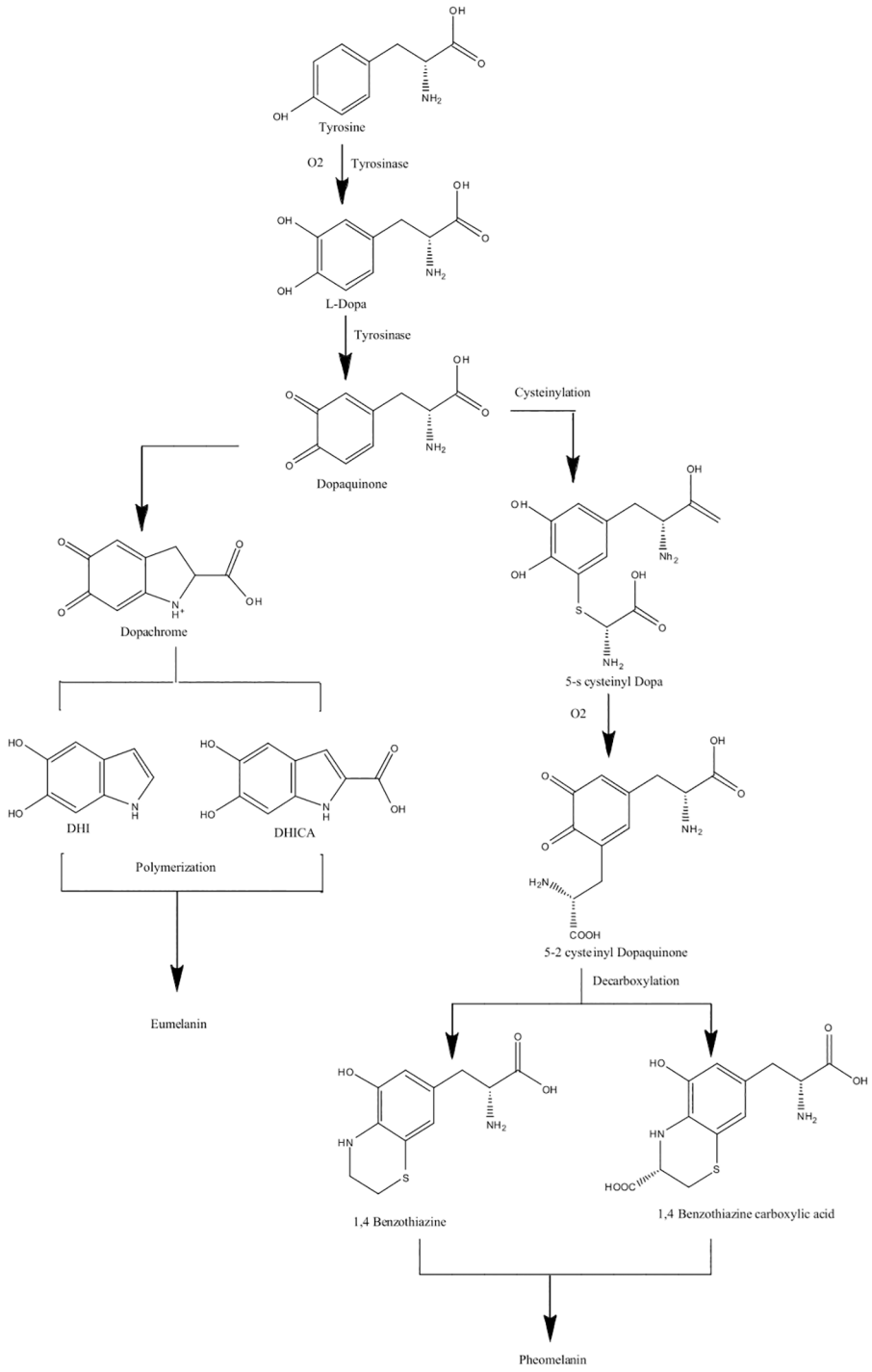

FIGURE 3
Biosynthesis pathway of eumelanin and pheomelanin (L-DOPA melanins) using tyrosinase enzymes. Pathways adapted from Suthar et al. (2023).

trihydroxynaphthalene to vermelone. Vermelone is then dehydrated by a multicopper oxidase gene *abr1*, which converts it to 1,8-dihydroxynaphthalene (1,8-DHN), which is polymerized into 1,8-DHN melanin by a laccase encoded by the putative laccase *abr2* gene (Perez-Cuesta et al., 2020). Various studies have looked at the functions of the genes involved in the 1,8-DHN pathway, and the results for some of them are summarized in Table 1.

5 Benefits of melanin

Melanin provides various benefits to organisms that produce it. One key aspect that distinguishes melanin from other natural chromophores is its ability to absorb every wavelength of light (Riesz et al., 2006; Cordero et al., 2018). Melanin is not essential for the growth of fungi, but it does facilitate the ability to survive

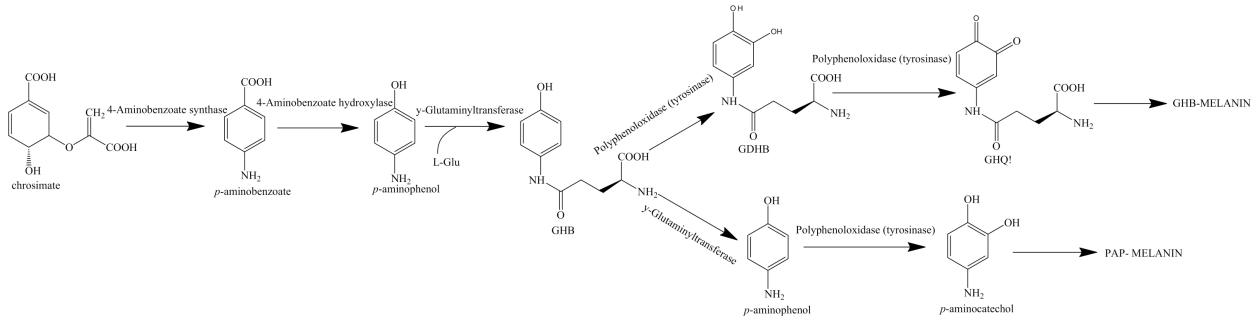

FIGURE 4
Biosynthesis pathway for GHB melanin using chorismate as a precursor. Pathway adapted from Weijin et al. (2013).

TABLE 1 Genes involved in fungal melanin biosynthesis.

Gene	Protein	Organism	Function	Reference
PKS/alb1 *PKS 12* and *PKS 13* *WdPKS1*	Polyketide Synthase	*A. fumigatus* *B. cinerea* *E. dermatitidis*	Production of 1,3,6,8-tetrahydroxynaphthalene (T4HN)	Gao et al., 2022 Schumacher, 2016 Paolo et al., 2006
Ayg1 *BRN1* and *BRN2*	Abhydrolase	*A. fumigatus* *M. laxa*, *M. fructicola*, *M.fructigena*	Reduction of 1,3,6,8 THN to scytalone	Gao et al., 2022 Verde-Yanez et al., 2023
arp1 *AISCD1* and *AISCD2* *SCD1*	Scytalone dehydratase	*A. fumigatus* *Ascochyta lentis* *M. laxa, M. laxa, M. fructicola*, *M. fructigena*	*Reduction of scytalone to 1,3,8-THN*	Gao et al., 2022 Debler and Henares, 2020 Verde-Yanez et al., 2023
arp2	Hydroxynaphthalene reductase	*A. fumigatus*	*Reduction of 1,3,8-THN to vermelone*	Gao et al., 2022
abr1	Vermelone dehydratase	*A. fumigatus*	Vermelone converted to 1,8-DHN	Gao et al., 2022 Upadhyay et al., 2013
abr2 *pbrB*	Oxydase	*A. fumigatus* *Talaromyces marneffei*	Polymerization of 1,8-DHN to 1,8-DHN melanin	Gao et al., 2022 Upadhyay et al., 2013 Sapmak et al., 2015
Tat	Tyrosine aminotransferase	*A. fumigatus*	Converts tyrosine to 4-hydroxyphenylpyruvate	Schmaler-Ripcke et al., 2009 Keller et al., 2011
hppD	4-hydroxyphenylpyruvate dioxygenase	*A. fumigatus* *A. niger*	Catalyzes precursor of pyomelanin, HGA	Schmaler-Ripcke et al., 2009 Koch et al., 2023
hmgA	Homogentisate dioxygenase	*A. fumigatus* *A. niger*	Degrades HGA to 4-Maleyl acetoacetate	Schmaler-Ripcke et al., 2009 Koch et al., 2023
fahA	Fumarylacetoacetate hydrolase	*A. fumigatus*	Degrades HGA to Fumarate	Heinekamp et al., 2013
maiA	Maleylacetoacetate isomerase	*A. fumigatus*	Degrades HGA to Acetoacetate	Heinekamp et al., 2013
melC2	Tyrosinase	*Streptomyces lincolnensis*	oxidize L-DOPA to generate melanin	Endo et al., 2001

TABLE 2 Benefits of melanin in fungi.

Benefits of Melanin in fungi	Reference
Protection from UV stress	Natarajan et al., 2014
Protection from various environmental stresses in fungi	Salgado-Castillo et al., 2023
Protection against microbes in fungi	Nosanchuk and Casadevall, 2003
Protection against oxidative stress	Rosas and Casadevall, 2006; Gorbushina et al., 2008; Kejžar et al., 2013; Cordero et al., 2018; Meredith and Sarna, 2006
Protection against enzymatic lysis	Rosas and Casadevall, 2001; Lin and Chen, 2005
Protection against radiation	Gessler et al., 2014; Pacelli et al., 2017; El-Bialy et al., 2019;
Provides structural support to the appressorium in plant pathogenic fungi	Belozerskaya et al., 2016; Chethana et al., 2021; Li et al., 2022

harmful conditions. The various benefits of melanin are reviewed in detail elsewhere (Cordero and Casadevall, 2017; Suthar et al., 2023) and summarized in Table 2.

6 Applied uses of fungal melanin

For industrial use, eumelanins are preferred over allomelanins, since allomelanins are attached to the inner side of the fungal cell wall, making the extraction process more challenging (Mattoon et al., 2021). Over the years, extraction protocols have been modified, resulting in significant improvements in melanin yields. Examples include *Auricularia auricula*, where 10% of the biomass consisted of melanin following treatment with lytic enzymes, guanidinium thiocyanate, chloroform, and HCl (Prados-Rosales et al., 2015), and *Armillaria cepistipes*, in which a 99% yield increase (27.98 g/L) was obtained using simpler extraction procedures (Ribera et al., 2019). Besides extraction protocols, nutrient composition, temperature, and pH have also been shown to play a part in melanin yield (Qin and Xia, 2024). Recent studies have also examined the overexpression of tyrosinase genes and their impact on melanin production (Tran-Ly et al., 2020; Mattoon et al., 2021). Other recent studies have also identified new *Exophiala* species that excrete melanin, thereby simplifying the extraction process (Carr et al., 2023). Ultimately, insights into the regulatory pathways that modulate melanin production in response to external factors will likely lead to the development of improved protocols for extraction as well as enhanced yields.

Extracted melanin molecules possess a range of potentially useful applications (Table 3). For example, their ability to absorb and dissipate photons from ionizing radiation highlights the potential value of melanin as a sunscreen (Wolbarsht et al., 1981). It has been proposed that due to UV absorption and cytotoxic activities of melanin, they also have therapeutic potential in cancer patients during radiation and chemotherapy treatments (Table 3). With an increased consumer demand for natural ingredients in food, melanin has been considered as a natural food coloring. Because the coloration of melanin can range from brown/black (i.e., eumelanin, allomelanin) to red/yellow (i.e., pheomelanin), these melanin molecules could be supplemented in food products as natural color agents instead of synthetic colors (Table 3) (Poorniammal et al., 2021; Yang et al., 2023). Various studies have also looked at the potential of melanin as an industrial coating for some packaging materials (Table 3).

TABLE 3 Potential uses of fungal melanin.

Species	Applied use	Reference
Agaricus bisporus	Protection from UV B	Olaizola et al., 2012
Agaricus bisporus	Packaging material	Łopusiewicz et al., 2018a
Agaricus bisporus	Antioxidant activity	Łopusiewicz et al., 2018b
Amorphothe caresinae	Absorption of both UV A And UV B	Oh et al., 2021
Aspergillus carbonarius	Potential yellow food coloring	Narendrababu and Shishupala, 2017
Auricula auricila-judae	Protection from ionizing radiation	Revskaya et al., 2012
Auricularia auricular	Reduced oxidative stress in mouse liver	Hou et al., 2021
Blakeslea trispora	Potential source of β-carotene	Nabae et al., 2005
Cryptococcus antareticus	Survival and low mutation rate from space radiation	Onofri et al., 2020
Gliocephalotrichum simplex	Reduced oxidative stress in mouse liver	Kunwar et al., 2012
Monascus purpureus SM001	Red food coloring	Blanc et al., 1995
Penicillium aculeatum	Anticancer drug	Krishnamurthy et al., 2020
Penicillium europium	Pink food coloring	Khan et al., 2021
Talaromyces purpureogenus	Yellow food coloring	Pandit et al., 2020
Thermomyces sp	Food coloring and antioxidant	Poorniammal et al., 2021
Trichoderma viride	Brown color pigment	Chitale et al., 2012

7 Regulation of melanin synthesis

Although the biosynthetic pathways of melanin production are relatively well known in fungi, less is understood about the regulation of these pathways. However, increasing evidence suggests that the PKA and HOG signaling pathways play a key role in the regulation of melanin production. The PKA-mediated cAMP signaling pathway is highly conserved among fungi, and the regulation of factors involved in virulence via cAMP is quite common (Langfelder et al., 2003; Alspaugh, 2015; Esher et al.,

2018). A study by Alspaugh et al. (1997) identified the role of the cAMP-dependent signaling pathway on melanin regulation in *C. neoformans*. A mutant strain with a defect in the Gpa1 Gα-protein, which showed reduced virulence and an inability to synthesize melanin, could be partially complemented by the addition of extracellular 3,5-cyclic adenosine monophosphate (cAMP) (Alspaugh et al., 1997, 1998). This was followed by a study by D'Souza et al. (2001), where the deletion of *pkr1* and *pka1* (two genes encoding the regulatory and catalytic subunits of PKA, respectively) produced avirulent *C. neoformans* strains that lacked melanin production. Other studies highlight a relationship between cAMP signaling and melanin production in plant pathogenic fungi, such as in *Ustilago hordei*, where high levels of cAMP inhibited melanin formation (Lichter and Mills, 1998). Various studies have documented the relationship between the cAMP/PKA pathway and virulence in *C. neoformans* (reviewed by Caza and Kronstad, 2019). In both *Magnaporthe oryzae* and *C. lagenarium*, cAMP signaling is involved in appressoria formation, which uses melanin to facilitate mechanical penetration of the host cell surface during infection (Adachi and Hamer, 1998; Takano et al., 2001). Various studies have also confirmed the importance of the cAMP/PKA signal transduction pathway and its involvement in the expression of genes involved in melanin biosynthesis (Brakhage and Liebmann, 2005; Yu et al., 2017).

A link between glycolysis and melanin production mediated by cAMP/PKA activation in fungi has also been established using mutant strains with defects in genes encoding phosphoglucose isomerase Pgil1 and trehalose synthesis (*TPS1* and *TPS2*) in both *C. neoformans* and *C. gattii* (Ngamskulrungroj et al., 2009; Zhang et al., 2015). These mutants had impaired cAMP/PKA activation with downstream effects on melanin production and the formation of the extracellular capsule (polysaccharide-based capsule). Further evidence of cAMP/pathway involvement was provided by Pukkila-Worley et al. (2005), where *gpa1* mutants had a negative impact on melanin and capsule production in *C. neoformans*, and *cac1* (adenylyl cyclase Cac1) mutants failed to produce melanin and capsules (Choi et al., 2015).

A study looking at transcription factors (TFs) in *C. neoformans* identified four melanin-regulating TFs—*Bzp4*, *Usv101*, *Mbs1*, and *Hob1*—that are required for the induction of the laccase gene (*LAC1*) (Lee et al., 2019). The study found that the cAMP pathway is not involved in the regulation of these four TFs, but the high osmolarity glycerol (HOG) response pathway has a negative impact on the induction of *BZP4* and *LAC1*. The study also focused on various protein kinases and identified Gsk3 and Kic1 deletion mutants as having a negative impact on LAC1 induction (Lee et al., 2019). Overall, this is the most comprehensive study that links specific transcription factors involved in the regulation of melanin synthesis to their cognate upstream signaling pathways.

A recent study determined the effects of protein kinase A (PKA) on *Candida auris* melanization. By performing gene deletion experiments, it was observed that the catalytic subunits Tpk1 and Tpk2 of PKA are important for *C. auris* melanization, whereas Ras1, Gpr1, Gpa2, and Cyr1 are not. Both *tpk1*Δ and *tpk2*Δ mutant strains formed melanin granules, but these melanin granules failed to adhere to the cell wall (Kim and Bahn, 2023). This study showed the importance of PKA catalytic subunits Tpk1 and Tpk2 in the control of chitin synthesis-related genes that are important for melanin granules to adhere to the cell wall. Since the melanin granules are not strongly associated with the cell wall, *C. auris tpk1*Δ *tpk2*Δ mutant strains were more susceptible to oxidative stress compared to the wild-type strain, where both strains produced similar melanin (Kim and Bahn, 2023).

In fungi, mitogen-activated protein kinase (MAPK) signaling pathways play a critical role in many cellular processes, including melanin biosynthesis by perceiving and responding to a variety of stresses or inputs (Gustin et al., 1998). Initial studies identified five MAPK pathways in *Saccharomyces cerevisiae* that were activated by different stimuli (Gustin et al., 1998; Saito, 2010). The orthologs of these five MAPKs have also been determined to play critical roles in different fungi (Turrà et al., 2014). Membrane-spanning proteins such as Sho1, Msb2, Hkr1, Opy2, Sln1, and Ste2 are conserved in fungi and function as sensors that detect stimuli such as osmotic stress, oxidative stress, nutrients, cell wall defects, mating signals, and developmental factors (Turrà et al., 2014; Kou and Naqvi, 2016). In various fungi, the high osmolarity sensitive sensors (i.e., Sho1) activate the HOG-MAPK signaling pathway in response to osmotic stress. In *Verticillium dahliae*, the mutant *ΔSho1* strain showed a reduction in melanin accumulation, and the expression of six genes involved in melanin synthesis was significantly affected (Li et al., 2019).

Evidence of the HOG1 pathway negatively regulating the synthesis of melanin was observed in a study conducted on *C. neoformans* by Bahn et al. (2005), which showed that the *hog1*ΔA mutant strain enhanced capsule formation and had a significant increase in the production of melanin in the serotype A strain H99. To determine the impact of the HOG1 pathway and its relationship with Pka1, it was observed that deletion of the *HOG1* gene resulted in restoring or, in some instances, enhancing the production of melanin in the serotype A *pka1*Δ mutants, suggesting that *HOG1* negatively modulates a downstream target of Pka1 in controlling melanin synthesis (Bahn et al., 2005). A follow-up study by Bahn et al. (2007) demonstrated the effect of a single gene (*SSK2*) encoding an upstream MAPKKK element of the Pbs2-Hog1 MAPK pathway. Ssk2 is known to activate the MAPKK pbs2 via phosphorylation. The *ssk2*Δ mutant strain had an enhanced production of capsules and melanin like the *hog1*Δ mutant, indicating that Ssk2 functions as a key MAPKK controlling the Pbs2-Hog1 MAPK pathway in *C. neoformans* (Bahn et al., 2007).

8 Transcription factors regulating fungal melanin production

Recent studies have focused on identifying the TFs involved in the pathways that control melanin biosynthesis. These TFs can either work upstream or downstream of various fungal melanization pathways to influence the expression of genes implicated in melanin production. Shelest (2017) identified 80 TF

families in more than 200 fungal species using whole-genome annotation for TFs. Out of the 80 families of TFs, three (i.e., C6Zn clusters, C2H2-like Zn fingers, and homeodomain-like TFs) were generally more prevalent. A previous study conducted by Tsuji et al. (2000) demonstrated the effect of the TFs Cmr1p and Pig1p in *C. lagenarium* and *M. oryzae*, respectively. Both TFs contained C2H2 Zn finger and C6Zn cluster DNA-binding motifs, and deletion of the Zn cluster led to a complete loss of melanin production, whereas deletion of the C2H2 cluster led to reduced melanin production (Tsuji et al., 2000). The TFs involved in the melanization process tend to be conserved across fungi, making them ideal targets to study the process of melanin production.

Cmr1 and its homologs are an example of a TF that is conserved in most melanin-producing fungi. Specifically, it has been shown to regulate genes related to melanin biosynthesis by promoting the expression of the *PKS* gene clusters, thus impacting growth, development, stress response, and virulence in various fungi such as *C. lagenarium*, *M. oryzae*, *Cochliobolus heterostrophus*, *Bipolaris oryzae*, and *Alternaria alternata* (Tsuji et al., 2000; Eliahu et al., 2007; Kihara et al., 2008; Cho et al., 2012; Li et al., 2022). In some fungi, the *PKS* gene and the *CMR1* gene show phylogenetic patterns, suggesting they are subjected to co-evolution (Jia et al., 2021). Evidence of co-evolution (or functional dependency) of the *PKS* and *CMR1* genes has been provided by various knockout studies. In *Alternaria brassicicola*, the *Δamr1* (homolog of cmr1) mutants created melanin-deficient colonies that were more sensitive to UV light (Cho et al., 2012). In *V. dahliae*, both *VdCmr1* and *VdPKS1* were necessary for melanin production, and the *ΔVdCmr1* strain had a 50% reduction in survival when exposed to UV irradiation or high temperatures (40°C) (Wang et al., 2018). In *Botrytis cinerea*, Bcsmr1 was involved in the regulation of genes involved in melanogenesis, and deletion of *bscmr1* led to defects in sclerotial melanogenesis, and an increase in the expression of *bscmr1* led to the accumulation of melanin (Zhou et al., 2017; Schumacher, 2016). In *Setosphaeria turcica*, deletion of the StMR1, a homolog of CMR1, led to the production of lighter colonies, and qPCR analyses confirmed that deletion mutants had significantly decreased expression of six key genes involved in the 1,8-DHN melanin synthesis pathway (Zhang et al., 2022). Another study identified two TFs—Pmr1 (homolog of Cmr1) and Pmr2—that regulate melanin biosynthesis, conidia development, and secondary metabolism in *Pestalotiopsis microspora* (Zhou et al., 2022). The deletion mutant *Δpmr1* showed defects in conidial pigmentation, and the mutant *Δpmr2* had decreased conidial pigmentation (Zhou et al., 2022). In *A. alternata*, the TF Aa*cmrA*, a homolog of cmr1, is required for melanin biosynthesis and pathogenicity. Work on mutant strains *ΔAacmrA* showed severely decreased melanin production, and the mutant strains were more sensitive to oxidative stress and cell wall inhibitors compared to the wild-type strain (Fetzner et al., 2014; Li et al., 2022). These studies provided evidence of the potential co-evolution of the TF Cmr1 and its homologs in various fungi and their involvement in fungal melanization and their effect on PKS.

Besides Cmr1, various other TFs have been identified that play a key role in melanin biosynthesis, some of which are described below. Two genes encoding bHLH (*DevR*) and MADS-box (*Rlm*A) TFs were identified in *A. fumigatus* located upstream of the melanin gene cluster acting as both a repressor and activator of the *pksP* promoter region to modulate the production of conidial melanin (Valiante et al., 2016). Another study identified two TF genes, *PfmaH* and *PfmaF*, that are part of the 1,8-DHN melanin biosynthetic gene cluster (*Pfma*) in *Pestalotiopsis fici* (Zhang et al., 2019). These studies showed that deleting the *PfmaF* did not affect melanin production, but overexpression of *PfmaF* led to heavy pigment accumulation in *P. fici* hyphae (Zhang et al., 2019). In *V. dahliae*, the TF VdMRTF1 is a bZip (basic leucine zipper domain) transcription factor that negatively regulates melanin biosynthesis (Lai et al., 2022). Transcriptomic analysis showed that VdMRTF1 regulates the expression of genes associated with melanin biosynthesis, tyrosine metabolism, and oxidative activity in *V. dahliae* (Lai et al., 2022). Besides BcSMR1, two other TFs—BcZTF1 and BcZTF2—are involved in the regulation of genes involved in melanogenesis in *B. cinerea* (Schumacher, 2016). Overexpression of *bcztf1* and *bcztf2* led to the accumulation of pigmentation in young mycelia, and the deletion mutants *Δbcztf1* and *Δbcztf2* led to colonies appearing white (Schumacher, 2016).

In *C. neoformans*, a GATA-type zinc finger TF (Cir1), which is involved in cAMP/PKA pathway regulation, also showed involvement in melanin and capsule formation (Jung et al., 2006). In another study, Cir1 was shown to regulate two genes involved in the HOG pathway, which is involved in capsule regulation (Haynes et al., 2011). In *C. neoformans*, four TFs (Bzp4, Usv101, Mbs1, and Hob1) were shown to be required for the induction of the laccase gene (*LAC1*) (Lee et al., 2019). Laccases have been shown to play a key role in both 1,8-DHN and L-DOPA melanin (Upadhyay et al., 2013). Another study that demonstrated both the effects of TFs and MAPks was performed on *C. heterostrophus*. In this study, it was found that two mitogen-activated protein kinases (Chk1 and Mps1) were important for normal melanin production (Eliahu et al., 2007). The mutant strains *Δchk* and *Δmps1* both produced white colonies and showed an autolytic appearance. Besides *Δchk* and *Δmps1*, deletion of the CMR1 TFs also resulted in albino mutants and the acquisition of orange-pink coloration, indicating the presence of other carotenoids or secondary metabolites besides melanin in *C. heterostrophus* (Eliahu et al., 2007). Table 4 summarizes various TFs in fungi and their impact on fungal melanization.

9 Future prospective

Melanin as a biomolecule has been known for over 150 years. Most studies on melanin have focused on its roles in virulence and pathogens that infect humans, animals, and plants. Other research has highlighted the benefits of melanin to fungi, such as protection from environmental stress, which enables them to grow in harsh environments. Melanin pigments are complex polymers whose diversity has made it challenging to investigate their structural properties. However, better extraction protocols coupled with the identification of genes and factors involved in the regulation of melanin biosynthesis have enabled researchers to gain a better

TABLE 4 Transcription factors (TFs) involved in melanin biosynthesis in various melanized fungi.

Transcription factor (TFs)	Impact	Type of melanin	Species	Reference
DevR RlmA	Production of conidial melanin	1,8-DHN	*A. fumigatus*	Valiante et al., 2016
Cmr1/PIG1/BMR1/Amr1 /CmMR1	Regulate melanin biosynthesis	1,8-DHN 1,8-DHN 1,8-DHN 1,8-DHN 1,8-DHN	*C. lagenarium* *M. oryzae* *B. oryzae* *A. brassicicola* *C. minitans*	Eliahu et al., 2007 Tsuji et al., 2000 Kihara et al., 2008 Cho et al., 2012 Luo et al., 2018
PfmaF	Overexpression leads to pigment accumulation	1,8-DHN	*P. fici*	Zhang et al., 2019
Pmr1 and Pmr2	Melanin biosynthesis/ conidia development	1,8-DHN	*P. microspora*	Zhou et al., 2022
AacmrA	Melanin production	1,8-DHN	*A. alternana*	Li et al., 2022
VdCmr1	Melanin biosynthesis/ pigment production	1,8-DHN	*V. dahliae*	Wang et al., 2018
VdMRTF1	Negative melanin regulation	1,8-DHN	*V. dahliae*	Lai et al., 2022
mtf1 and mtf2	Upregulation of PKSs genes	1,8-DHN	*U. maydis*	Reyes-Fernandez et al., 2019
BcSMR1, BcZTF1 and BcZTF2	Regulation of gene involved in melanogenesis	1,8-DHN	*B. cinerea*	Schumacher, 2016
Bzp4, Usv101, Mbs1 and Hob1	Induction of laccase gene (*LAC1*)	L-DOPA	*C. neoformans*	Lee et al., 2019
Cir1	Melanin deposition in the cell wall	L-DOPA	*C. neoformans*	Jung et al., 2006
VdZFP1 and VdZFP2	Involved in melanin deposition	1,8-DHN	*V. dahliae*	Li et al., 2023
Vta1/ VdpF	Involved in melanized microsclerotia development	1,8-DHN	*V. dahliae*	Harting et al., 2020

understanding of fungal melanization. The biosynthetic pathways for 1,8-DHN melanin and L-DOPA melanin are well known, but recent focus has shifted toward identifying the various TFs and signaling pathways that regulate production. TFs can act as either an activator or a repressor depending on the context in which they bind to their target DNA. Recent work has also provided insight into the regulation of melanin by the cAMP/PKA and the MAPK Hog1 pathways, including links between these pathways and downstream TFs (Cordero et al., 2020; Lee et al., 2019). The level of complexity underlying these links in just one fungus (i.e., *C. neoformans*) suggests that comparable systems-level studies are needed in other fungi such as *E. dermatitidis* to determine the extent, if any, to which regulatory features are conserved.

A more robust mechanistic understanding of the signaling pathways and TFs involved in fungal melanization will help in harnessing the potential benefits of melanin as bio-based components of sunscreens, natural food coloring agents, and packaging materials. These applications have generated more interest in understanding regulatory pathways that can be manipulated to increase the concentration of melanin. In addition, melanin biosynthetic pathways produce many intermediates that have different properties. By genetically modifying strains to hamper or enhance the production of certain intermediates in these biosynthetic pathways, the extraction and yield of the beneficial intermediates can be increased. Another way to increase melanin yield is to genetically modify strains with overexpression of tyrosinases or laccases that play a key role in melanin production. Since melanin production is affected by environmental conditions, experimenting with nutrient composition and growing conditions such as pH, temperature, and aeration can impact the yield of melanin as well.

At this time, it is fair to assume that additional pathways that regulate melanin production beyond those already known remain to be discovered. Systems-level studies that leverage new genetic and genomic-based resources in emerging polyextremotolerant fungi (Erdmann et al., 2022; Carr et al., 2025; Colarusso et al., 2025) represent a promising approach toward addressing this challenge, as do studies that combine classical genetics with genome resequencing (Chhoker et al., 2025). Insights generated by such studies would provide a much more comprehensive understanding of how fungi coordinate melanin production with specific environmental inputs. For example, in those fungi capable of producing multiple types of melanin, do specific inputs direct the synthesis of a particular type of melanin? Obvious benefits derived from these insights include enhanced capacities to engineer melanin production for specific applied purposes. Moreover, they would also create opportunities to delve into broader evolutionary questions regarding the role(s) that the regulation of melanin synthesis might have played in facilitating the adaptation of polyextremotolerant fungi to harsh environmental niches.

Author contributions

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