

Melanins in fungi: Types, localization and putative biological roles



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ARTICLE INFO

Article history:

Received 2 January 2017

Received in revised form

6 April 2017

Accepted 6 April 2017

Available online 8 April 2017

Keywords:

Melanins-stress-wall deposition-synthesis

ABSTRACT

Melanin is a secondary metabolite made up by complex heterogeneous polymers. Several classes of fungal melanins have been described like γ -glutaminy-3,4-dihydroxy-benzene (GDHB)-, L-DOPA-(eumelanin), DHN-, catechol-melanin, pyromelanin, *p*-aminophenol (PAP)-melanin, heterogeneous melanins as well as two others based on 5-deoxybostrycoidin and aspulvinone E. They have been mostly localized in cell walls of different structures of fungi, which suggest that they play a different biological role. They have been related mostly with morphogenesis, stress resistance, virulence and energy transduction. However, an enormous amount of work remains to be done to clarify, among other things, the role melanins play in fungi.

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1. What do we define as melanin?

Melanins are secondary metabolites made up of complex heterogeneous polymers of phenolic and/or indolic monomers. Recently, nuclear magnetic resonance (NMR) studies showed that melanins area network of aliphatic and aromatic structures. Also, Sun et al. [1] by 2D-NMR spectroscopy and ¹H-¹³C heteronuclear single quantum correlation (HSQC), found in the crude extracellular melanin of *Auricularia auricula*, signals of both aliphatic and aromatic structures. Therefore the condensed molecular formula of

melanin might be $[C_{18} (OR)_3 H_7 O_4 N_2]_n$.

2. Melanins synthesis and localization in fungi

Melanins structure increases in complexity as fungal cultures grew older. These pigments have carbon-containing covalent linkages between aromatics and either chitin-derived *N*-acetylglucosamines or membrane glycerides. Solid-state NMR data of the melanin of *Cryptococcus neoformans* support a stacking of indol-based aromatic structures through covalent cross-linkages, a material that is ordered at microsites but globally amorphous [2]. Recently, Beltrán-García et al. [3] reported that the melanin isolated and purified from the mycelium of *Mycosphaerella fijiensis* is constituted by 50 units of 1,8-dihydroxynaphthalene (DHN). Since

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there is a lack of knowledge regarding the structure of other types of melanins additional studies should be performed in order to know more about their structure. Further analysis, using solid-state NMR and physical/imaging techniques, should provide the bases to characterize the pigment architecture in the cell wall, which will be useful to disregard artifacts derived from the harsh chemical treatments that are frequently used to extract DHN-melanin.

Frequently, soluble melanins such as pyomelanin and heterogeneous melanins as well as insoluble ones, mostly immobilized in the cell wall, also are released in culture supernatants. In *Candida albicans* and *Cryptococcus neoformans* melanins are extruded that seems to occur through a complex set of events that include vesicle-like structures that are free or often loosely bound to the cell wall exterior [2,4]. However, melanins deposition and localization in the cell wall of fungi vary within species, with culture conditions and/or the structures differentiated by each fungus [4]. This basically suggests that in fungi melanin might be playing more than just one biological role. Future studies should be aimed not only at understanding their biological role but also at clarifying the way melanins are secreted in other fungi.

While in some fungi melanin is part of a layer tightly associated to the plasma membrane on the inner side of the cell wall, like in the appressorium of *Magnaporthe grisea*, in other fungi like in *Cryptococcus neoformans*, it is deposited throughout the cell wall in the form of concentric rings. Alternatively melanin is deposited in the outermost layers such as in the hyphae of *Pseudocercospora griseola* or as granules on the surface of the walls of pseudohyphae and yeasts like *Candida albicans* [4–6].

Melanin is synthesized and secreted in specialized vesicles, occasionally denominated 'virulence bags', in an analogous way to the mammalian melanosomes [7]. In this way, the extracellular polymer is transported out of the cell and can be anchored to the wall. Therefore, most probably the environmental conditions as well as the metabolic activity of fungi might alter melanin synthesis and extrusion in different fungi.

Many fungi contain melanins in vegetative as well as reproductive structures such as those belonging to the dematiaceous fungi that are known as the brown-pigmented ones. Melanins such as 1,8-DHN- and L-3,4-dihydroxyphenylalanine (L-DOPA)-ones have been mostly associated to ascomycetous and basidiomycetous ones, respectively. However, some representative fungi from both taxonomic groups (phyla), such as those of a genus, can synthesize different dark pigments. Furthermore in many cases they produce pigments in specific structures. In this sense, some fungi have the ability to synthesize a type of melanin according to the environmental conditions as well as the developmental stage (Table 1). The presence of melanins in fungi adapted to different environments and the fact that the same melanin is synthesized by a fungus, independently of the environmental conditions, suggests that this molecule plays different crucial biological roles in nature. However, more studies should be performed at identifying the role that different types of melanins play on biological processes.

3. Types of melanins synthesized by fungi

While many fungi synthesize melanins constitutively, others do so only under certain culture conditions and/or if specific precursors are available. While several fungi synthesize melanin endogenously via a DHN intermediate, others, like *Tetraploa aristata* [32], synthesize an undefined set of soluble black pigments that are known as heterogeneous melanins. They are produced by a mechanism of detoxification that is responsible of polymerization of phenolic compounds of various origins, including those present in fungal secretions, which are available outside the cell [33].

Based on their biochemical precursors, pathways and/or

components several classes of fungal melanins have been described like γ -glutaminy-3,4-dihydroxy-benzene (GDHB)-,L-DOPA-, which is known as eumelanin, DHN-, catechol-melanin, pyomelanin and *p*-aminophenol (PAP)-melanin as well as the heterogeneous melanins. Recently, two new melanins have been reported. One is located within the periderm of perithecia of representatives of the *Fusarium graminearum* group, it is based on 5-deoxybostrycoidin, which is synthesized from the reaction of anhydrofusarubin derivatives and ammonia [20]. The other one is Asp-melanin that is present in the wall of conidia of *Aspergillus terreus*. The precursor of this melanin, 4-hydroxyphenylpyruvate, is oxidized generating in this way aspulvinone E derivatives, which are polymerized [11].

Fungal melanins as well as those of plants are and have been known as allomelanins, an unclear term that include dark pigments synthesized from nitrogen-free precursors, such as those derived from catechol and DHN [34]. Furthermore, if pigment synthesis includes the incorporation of cysteine into their L-DOPA-derived structure, like in *Auricularia auricula*, they are known as pheomelanins [1]. Although the polyketide synthase pathway is involved in the synthesis of DHN-melanins, also phenoloxidases have been related to its synthesis as well as in DOPA-melanin (Fig. 1). Interestingly, disruption of genes of several melanin-pigmented fungi resulted in mutants that developed albino colonies *in-vitro*. When they were complemented with wild type genes, the pigmented phenotype was recovered. Furthermore, the melanin found in conidia of *A. terreus* is synthesized by a non-canonical pathway, which includes an unusual non-ribosomal peptide synthase-like enzyme and a tyrosinase [11]. This is a peculiarity considering that Aspergilli mostly synthesize DHN-melanin through a polyketide synthase derived naphthopyrone. So the enzymes and mechanisms involved in the different pathways of melanin synthesis still remain obscure (Fig. 1).

4. Roles of melanins in fungi

Fungal melanins are unique in that they play a myriad of biological roles, in morphogenesis, virulence, energy transduction and/or C storage. However, in fungi melanins are scavengers of stresses, protecting in this way cell viability, which does not seem to be the only role.

In terms of morphogenesis, melanin was found to be a key structural component of conidial wall of *Aspergillus fumigatus*. Also, it has been related to the expression of adhesins as well as other virulence factors [35], therefore it appears that in this case melanin plays a critical role, very early, just before infection occurred. In addition to this in *Bipolaris sorokiniana* melanin has been associated to the development of aerial hyphae and conidiogenesis [14], suggesting that they might play another role. So melanins might affect fungi very early as well as late along their life cycle.

Melanins are required by many fungi like plant, animal and human pathogens and because of this have been associated with virulence. The radical and chemical nature of melanins suggests that they might be working as a shield against hydrolytic enzymes that may affect fungal cell walls. In addition to this, melanins also might sequester host defensive proteins and metals, trap single electrons or act as a photoactivated 'toxin' that generate singlet molecular oxygen, which has been shown for the DHN-melanin of *M. fijiensis*. In several plant pathogens melanin plays a pivotal role in generating osmotic pressure within the appressorium, when hyphae penetrate plant cell walls, which was described for *Magnaporthe* and *Colletotrichum* species. In these pathogens, mutations aimed at inhibiting melanization led to a reduction in virulence.

Melanins enhance tolerance of fungi to environmental stresses, improving in this way their survival. Melanins protect fungal structures from UV radiation, temperature, oxidizing agents and

Table 1
Type, localization and putative role of melanins in different fungi.

Taxa ^a	Melanin type	Localization	Putative role	Reference
<i>Agaricus bisporus</i> ^b (Agaricaceae)	GHB-	Basidiospore wall	?	[8,9]
	PAP-	?	?	
	DOPA-	?	?	
	Catechol-	?	?	
<i>Aspergillus terreus</i> ^a (Trichocomaceae)	Asp-	Conidia	Tolerance to UV radiation and acidic environments and defence	[10,11]
	DHN-	Culture broth	from predation by amoeba	
<i>Auricularia auricula</i> ^b (Auriculariaceae)	Pheomelanin	Fruiting bodies	?	[1,12,13]
	DHN-	Fruiting bodies	?	
	Eumelanin-type	Extracellular medium	?	
<i>Bipolaris sorokiniana</i> ^a (Pleosporaceae)	DHN-	Wall in hyphae and conidia	Development, fitness and survival	[14,15]
<i>Cenococcum geophilum</i> ^a (Gloniaceae, Mytiliniidiales)	DHN-	Hyphae wall	Tolerance to water stress	[16,17]
<i>Cladosporium cladosporioides</i> ^d (Cladosporiaceae)	DHN-melanin like compounds	Wall in hyphae and conidia	Tolerance to stresses imposed by fungicides or the environment	[18]
<i>Cryptococcus neoformans</i> ^b (Tremellaceae)	DOPA- (eumelanin)	Extracellular vesicles and cell wall	Pathogenicity. Virulence factor. Protection against host defence systems (immune effector cells, phagocytosis and oxidative injury)	[7,19]
<i>Fusarium graminearum</i> ^a (Nectriaceae)	5-Deoxybostrycoidin-	Perithecial wall	Tolerance to UV radiation and reactive oxygen species	[20]
<i>Metarhizium anisopliae</i> ^a (Clavicipitaceae)	DHN-	?	Virulence factor. Hydrophobic attraction, water absorption, adhesion, conidia germination and tolerance to water and oxidative stress	[21]
<i>Monilinia laxa</i> ^a (Sclerotiniaceae)	DHN-	All structures differentiated	Pathogenicity. Sporogenesis. Survival. Tolerance to environmental stresses and autolysis	[22]
<i>Mucor</i> spp. ^d (Mucorales)	?	Outer wall of sporangiospores	?	[23]
<i>Neocallimastix</i> sp. ^e Isolate MC-2 (Neocallimastigaceae)	?	Wall of mature resistant sporangia	Survival	[24]
<i>Ophiocordyceps sinensis</i> ^a (Ophiocordycipitaceae)	DOPA-	Fermentation broth of submerged cultures	?	[25]
<i>Penicillium chrysogenum</i> ^a (Trichocomaceae)	Pyomelanin	Extracellular medium	Survival under nutrient and water stress	[26]
<i>Pseudocercospora griseola</i> ^a (Mycosphaerellaceae)	DHN-	Wall in hyphae and conidia	Morphogenesis	[5,27]
<i>Sporothrix schenckii</i> ^a (Ophiostomataceae)	DHN-	Constitutively in conidial and yeast cells	Virulence related to infection and against phagocytosis	[28–30]
	DOPA-(eumelanin)	Wall of conidia, yeast cells, and hyphae if	Virulence related to infection and against phagocytosis. Protection against antifungal drugs	
	Pyomelanin	Extracellular medium if L-tyrosine is available during fungal growth	Quencher of oxidative stress. Protection against antifungal drugs	
<i>Synchytrium endobioticum</i> ^c (Synchytriaceae, Chytridiales)	Allomelanin like compounds ?	Resting spores	Survival	[31]

^aa, phylum Ascomycota; ^bb, phylum Basidiomycota; ^cc, phylum Chytridiomycota; ^dd, subphylum Mucoromycotina; ^ee, phylum Neocallimastigomycota. GHB, γ -L-Glutaminyl-4-hydroxybenzene. PAP, *p*-Aminophenol. DOPA, L-3,4-dihydroxyphenylalanine. DHN, 1,8-dihydroxynaphthalene.

antibiotics [36]. Melanins might be playing the role of an extracellular redox buffer that can neutralize oxidants generated by environmental stresses. Furthermore, melanins protect fungi from solar radiation and UV light. In fact, pigments dissipate light energy either as heat or in chemical reactions or by scavenging active oxygen species, e.g. superoxide, and singlet oxygen, which result in the consumption of molecular oxygen. Therefore, melanin protects fungi against a broad range of environmental stresses as well as toxic molecules, improving in this way their survival ability. However, the protective role each melanin plays in nature is dependent on its synthesis pathway, its localization within cells as well as its behavior against an environmental stress. Just as an example, viability of *A. terreus* conidia within acidic environments has been related to the presence of the Asp-melanin in the wall, suggesting that the pigment plays an ecological role [11].

Melanized fungal cell walls are relatively resistant to decomposition; because of this melanins might be a source of storage C in the soil. Organic matter derived from *Cenococcum geophilum*, which is drought tolerant and abundant in water-stressed habitats, may represent as much as 50% of organic C in soils [16]. Dark pigments like melanins might also function in energy transduction, which

might enhance growth of melanized fungi also suggesting a role for capturing and utilizing energy [37].

The broad spectrum of functions fungal melanins play suggests that they might have several applications in medicine as well as in pharmacology, cosmetics and/or other fields [25]. The knowledge of the biosynthesis as well as the role melanins play in pathogenic fungi as well as the enzymes involved in melanization is crucial to design strategies to manage plant pathogens of crops.

5. Final considerations

Data regarding the structural organization of melanins in fungi, whether they are located in cell walls or elsewhere, including the extracellular medium, are scarce. Such studies have only been performed in *Candida albicans* and *Cryptococcus neoformans*, therefore there is no information regarding the mechanisms involved in melanization in other fungi. It is known that while some fungi synthesize one type of melanin, others synthesize two or three different ones. This suggests either that each one plays a different biological role or that their crucial role oblige organisms to have alternative biosynthetic pathways so that they are available. In

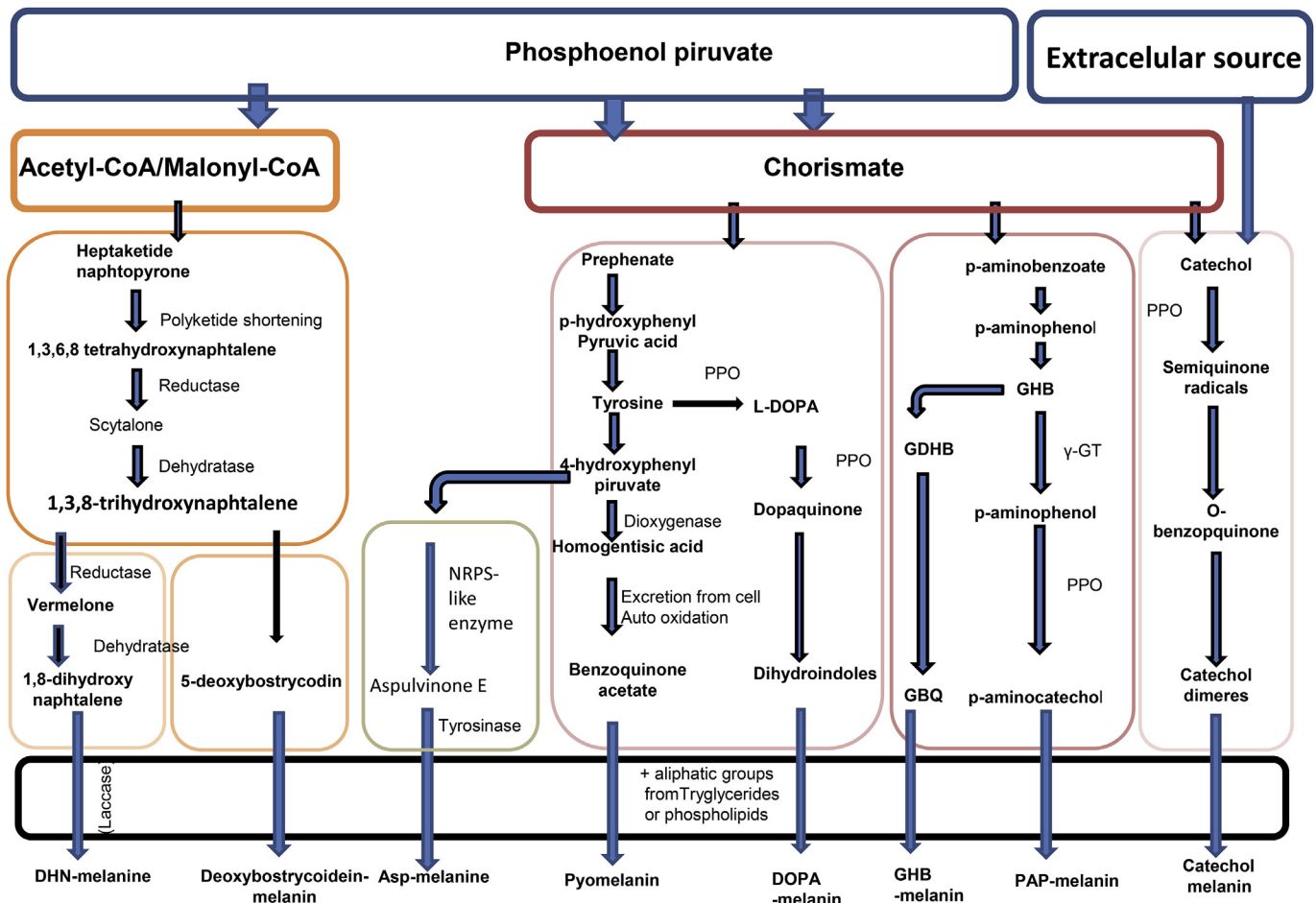


Fig. 1. Pathways for the synthesis of different types of melanins in fungi. PPO, polyphenol oxidase. GHB, γ -L-glutaminy-4-hydroxybenzene. GDHB, γ -L-glutaminy-3,4-dihydroxybenzene. γ -GT, γ -Glutaminytransferase. GBQ, γ -L-glutaminy-3,4-benzoquinone. NRPS, non-ribosomal peptide synthase.

line with this, Frandsen et al. [20] found that in *Fusarium* pigmentation is sustained by conservation, replacement and development of redundant pigments. However, different melanins have been found in fungal structures, which can be additionally altered by the environment that also might differentially regulate their synthesis. All this together strongly suggests that melanins play several crucial roles in fungi mostly related with survival and also that each type probably plays a specific biological role.

It is also important to consider that the same melanins are present in phylogenetically unrelated fungi as well as functionally diverse groups. As mentioned before this suggests that melanins play a similar function in diverse fungi and help all of them to cope with the stresses imposed by each environment. Also the fact that some fungi synthesize more than one melanin suggest either that each of them plays a different biological role quenching the effect of different damaging agents or that they are so important for survival of fungi that they synthesized more than just one type of them.

Additional studies should be performed aimed at clarifying the synthesis and role these pigments play in fungi such as in representatives of the Mucoraceae, Neocallimastigaceae and Synchytriaceae, which should be easier considering the availability of genomes of related fungi. This will allow performing a comparative analysis of genes coding for the enzymes involved in melanin synthesis as well as their expression levels under different environmental conditions. This should also be a complement with site directed mutations that together with phenotype evaluation and

complementation studies will allow researchers to elucidate the specific role each type of melanin play in fungi. These lines of research are obvious but even though researchers are aware of the importance of melanins in nature, their role in fungal biology remain unknown.

Financial support

This work was supported by PICT 2012-2760 and PICT 2015-1620 ANPCyT and grants provided by Comisión de Investigaciones Científicas de la Provincia de Buenos Aires and the Universidad Nacional de La Plata. AT and MCNS are researchers of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). MEEF and SMYL are fellows of CONICET. MIT is a fellow of the Universidad Nacional de La Plata. PAB is a researcher of the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICBA).

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