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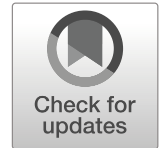
MINI-REVIEW



Biosynthesis of conidial and sclerotial pigments in *Aspergillus* species

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Abstract
Fungal pigments, which are classified as secondary metabolites, are polymerized products derived mostly from phenolic precursors with remarkable structural diversity. Pigments of conidia and sclerotia serve myriad functions. They provide tolerance against various environmental stresses such as ultraviolet light, oxidizing agents, and ionizing radiation. Some pigments even play a role in fungal pathogenesis. This review gathers available research and discusses current knowledge on the formation of conidial and sclerotial pigments in aspergilli. It examines organization of genes involved in pigment production, biosynthetic pathways, and biological functions and reevaluates some of the current dogma, especially with respect to the DHN-melanin pathway, on the production of these enigmatic polymers. A better understanding of the structure and biosynthesis of melanins and other pigments could facilitate strategies to mitigate fungal pathogenesis.

Keywords *Aspergillus* · Melanin · Pigment biosynthesis · Conidia · Sclerotia

Introduction

According to Catalogue of Life (https://www.catalogueoflife.org/), there are about 500 recognized *Aspergillus* species. Examples include the genetic model for the genus, *Aspergillus nidulans*; the primary pathogen for human invasive aspergillosis *Aspergillus fumigatus*; the lovastatin-producing *Aspergillus terreus*; the GRAS (generally regarded as safe) *Aspergillus oryzae* and *Aspergillus sojae* that are widely used in food fermentation; and aflatoxin-producing species such as *Aspergillus flavus* and *Aspergillus parasiticus* that negatively impact global food safety and economics. The genus *Aspergillus* is named for its distinct morphology, which resembles an aspergillum used by Catholic priests to sprinkle holy water. It consists of a conidiophore stipe terminating in a swollen vesicle that may bear one layer (uniseriate) of specialized cells called phialides, on which conidia (asexual spores) are borne (Klich 2002). Biseriate species have a layer of cells between the vesicle and phialides called metulae. In addition to producing asexual conidia, some aspergilli also produce sclerotia. They are resting structures formed by the aggregation of hyphae into discrete, non-pigmented initials that subsequently develop into dense, pigmented structures (Willetts and Bullock 1992). Sclerotia represent a major source of fungal propagules in the field that remain viable for long periods of time under adverse environmental conditions. Upon onset of favorable conditions, they germinate by producing hyphae that eventually form aerial conidiophores with conidiospores. Similar to conidia, sclerotia can harbor a number of secondary metabolites (Frisvad et al. 2014). In some heterothallic *Aspergillus* species such as *A. flavus*, *A. parasiticus*, and *Aspergillus nomius* (teleomorph in genus *Petromyces*), sclerotia (stromata) also play a role in sexual reproduction by containing many ascospore-bearing fruiting bodies, termed cleistothecia, following fertilization by a sexually compatible strain (Horn et al. 2016).

Like other fungi, *Aspergillus* species produce a variety of pigments. These pigments are often present in vegetative structures such as hyphae, reproductive spores such as conidia (asexual) and ascospores (sexual), and sclerotia. Often, these pigments are used to distinguish between species. Of the fungal pigments, melanins are the most studied but are highly recalcitrant to structural characterization. Both conidial and sclerotial pigments are considered melanins. Fungal melanins are high-molecular-weight amorphous substances formed from the oxidative

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polymerization of phenolic or indole products (Cordero and Casadevall 2017). It is commonly believed by many researchers that fungal melanins are synthesized from the polymerization of 1,8-dihydroxynaphthalene (DHN) or, alternatively, from the polymerization of L-3,4-dihydroxyphenylalanine (L-DOPA) (Eisenman and Casadevall 2012). However, this is not entirely true especially in the case of conidial pigments produced by aspergilli. Melanins enable fungi to cope with harsh environments and unfavorable growth conditions, providing protection against desiccation, ultraviolet light, ionizing radiation, and oxidative stress (Belozerskaya et al. 2015). In addition, they contribute to fungal pathogenesis, survival against phagocytosis, and longevity of fungal propagules (Bell and Wheeler 1986).

Organization of genes involved in conidial pigment biosynthesis

The advent of the genomics era has resulted in the availability of a large amount of genome sequence data. Although few fungal genomes have been assembled at the chromosomal level, available sequence contigs or scaffolds have allowed researchers to explore and confirm physical linkage of genes, especially those related to production of secondary metabolites. Genes involved in conidial pigment biosynthesis in *A. fumigatus* form a gene cluster (Tsai et al. 1999). However, this well-defined organization seems to be an exception rather than a norm. For *A. niger* and *A. flavus*, two of their pigment genes, namely, *olvA/aygl* (An14g05350) and *brnA* (An14g05370) of *A. niger* as well as *wA* (AFLA_006170) and *olgA* (AFLA_006180) of *A. flavus*, are adjacent to each other on a chromosome (Table 1), while other characterized conidial pigment biosynthetic genes are located on different chromosomes. This likely is also true for *A. oryzae* (Katayama et al. 2016; Machida et al. 2008), a species genetically closely related to *A. flavus*. In the case of *A. terreus*, two of its pigment genes, *melA* (ATEG_03563) and *tyrP* (ATEG_03564), are situated next to each other, but it is not known whether additional unidentified genes are required for conidial pigment biosynthesis. For *A. nidulans*, only *wA* (AN8209) (Watanabe et al. 1999) and *yA* (AN6635) (Mayorga and Timberlake 1990) have been characterized, and they are located on chromosome II and chromosome I, respectively. Table 1 summarizes currently known genes involved in conidial pigment biosynthesis in various aspergilli. For conidial pigment biosynthesis, gene orthologues encoding polyketide synthases (PKSs) seem to be commonly present in *Aspergillus* species, but pigment genes encoding nonribosomal-peptide synthetases (NRPS) or NRPS-like enzymes are rare. In addition to the *pks* genes, *A. fumigatus aygl* orthologues that encode the YWA1 hydrolase are often present. They include those orthologues confirmed in *A. fumigatus* and *A. niger* (Chiang et al. 2011; Jorgensen et al. 2011; Tsai et al. 2001) and other possible orthologous genes from *A. nidulans*, *A. flavus*, and *A. oryzae* (AO090005000332) based on information retrieved from the *Aspergillus* Genome Database, AspGD (http://www.aspergillusgenome.org/), and a previous review (Baker 2008). Depending on the pigment precursors, either tyrosinases or laccases are involved in subsequent polymerization steps that impart conidia their characteristic colors, which are routinely used as a criterion in species identification by mycologists.

The YWA1 precursor is commonly synthesized by specific polyketide synthases

Like the *A. nidulans wA* knockout mutant, the *A. fumigatus alb1* knockout mutant produces non-pigmented (white) conidia. Both *wA*- and *alb1*-encoded PKSs are responsible for the production of YWA1, a naphthopyrone (Fig. 1). Heterologous expression of the *pks* genes in *A. oryzae* or *A. terreus* also has confirmed YWA1 production (Slesiona et al. 2012; Watanabe et al. 2000). YWA1, a yellow metabolite, is the first precursor for the *A. nidulans* green pigment (Watanabe et al. 1999) and for the *A. fumigatus* bluish/grayish green pigment (Tsai et al. 2001) in respective mature conidia. The *A. niger albA*-encoded PKS also is responsible for the production of YWA1 and a family of naphthopyrones found in significant quantities in culture extracts (Chiang et al. 2011). Consistently, the *A. flavus wA* knockout mutant produces non-pigmented white conidia (Chang et al. 2010). Most recently, an *A. flavus* spontaneous mutant that produces yellow conidia because of a deletion in the copper-transporting ATPase gene specifically involved in conidial pigment biosynthesis has been isolated (Chang et al. 2019). This yellow pigment likely is YWA1, or a naphthopyrone analogue, that is not converted to downstream polymeric metabolites by the resulting nonfunctional laccases (apoenzymes) due to the deficiency in the intracellular copper ions necessary for the laccases' polymerization function. Parasperone A, a pigment structurally similar to YWA1, has been isolated from conidia of a laccase-deficient strain of *A. parasiticus* (Brown et al. 1993), a species genetically similar to *A. flavus*. YWA1 is also the precursor of aurofusarin, a red pigment found in mycelia and secreted into culture medium by *Fusarium graminearum* (Frandsen et al. 2011). However, despite the seemingly initial common step of naphthopyrone formation among these few aspergilli, other steps for conidial pigment biosynthesis appear to be species-dependent.

Table 1 Conidial pigment biosynthetic genes in *Aspergillus* species

	Gene IDs	Product	Conidial color	Chromosome[a]	Reference
A. fumigatus					
alb1	Afu2g17600	Polyketide synthase (1)[b]	White	II	Tsai et al. 1999
ayg1	Afu2g17550	α/β hydrolase (2)	Yellowish green	II	Tsai et al. 1999
arp2	Afu2g17560	T4HN reductase (3)	Reddish pink	II	Tsai et al. 1999
arp1	Afu2g17580	Scytalone dehydratase (4)	Reddish pink	II	Tsai et al. 1999
abr1	Afu2g17540	Laccase	Brown	II	Tsai et al. 1999
abr2	Afu2g17530	Laccase	Brown	II	Tsai et al. 1999
A. nidulans					
wA	AN8209	Polyketide synthase	White	II	Watanabe et al. 1999
yA	AN6635	Laccase	Yellow	I	Mayorga and Timberlake, 1990
A. niger					
fwnA	An09g05730	Polyketide synthase	Fawn		Jorgensen et al., 2011
brnA	An14g05370	Laccase	Brown		Jorgensen et al., 2011
olvA/ayg1	An14g05350	α/β hydrolase	Olive		Jorgensen et al., 2011
pptA	An12g03950	4′-phosphopantetheinyl transferase	White		Jorgensen et al., 2011
A. flavus					
wA	AFLA_006170	Polyketide synthase	White	IV	Chang et al. 2010
olgA	AFLA_006180	Laccase	Dark green	IV	Chang et al. 2010
ctpA	AFLA_051390	Copper-transporting ATPase	Yellow	I	Chang et al. 2019
gldA	AFLA_045660	Laccase	Gold	VII	Chang et al. 2019
pptA	AFLA_046430	4′-phosphopantetheinyl transferase	White	VII	Chang unpublished
A. oryzae					
wA	AO090102000545	Polyketide synthase	White	IV	Katayama et al. 2016
yA	AO090011000755	Laccase	Yellow	VII	Katayama et al. 2016
A. terreus					
melA	ATEG_03563	Nonribosomal peptide synthetase-like	White		Geib et al. 2016
tyrP	ATEG_03564	Tyrosinase	Fluorescent yellow		Geib et al. 2016
A. carbonarius					
alb1	172075	Polyketide synthase	Fawn		Gerin et al. 2018

[a] Chromosome locations were retrieved from the *Aspergillus* Genome Database (http://www.aspergillusgenome.org/)

[b] See Fig. 1 for enzymes involved in specific catalytic steps of DHN-melanin biosynthesis

Is the DHN-melanin pathway a major source of *Aspergillus* pigments?

The majority of *Aspergillus* species are believed to produce DHN-melanin (Jorgensen et al. 2011; Tsai et al. 1999) despite the lack of conclusive evidence from literature. This belief may have been extrapolated from the well-studied *A. fumigatus* conidial pigment biosynthesis. In this particular *A. fumigatus* pathway, Ayg1, an α/β hydrolase, converts the 14 carbon YWA1 to the pentaketide 1,3,6,8-tetrahydroxynaphthalene (T4HN) by releasing an acetoacetic acid from YWA1 at the same time (Fujii et al. 2004) (Fig. 1). Interestingly, the synthesis of T4HN in *Collectotrichum lagenarium* only requires a single *pks* gene (Watanabe and Ebizuka 2004), which suggests that the PKS is specific for the pentaketide synthesis without the need of a hydrolase. The *A. fumigatus ayg1* knockout mutant produces yellowish green conidia, in contrast to the bluish green conidia of the wild type, due to the accumulation of YWA1. The presence of DHN-melanin in an *A. fumigatus* environmental isolate has been confirmed by physico-chemical analyses; characteristic peaks in UV–Vis and IR spectra unique to DHN-melanin have been identified (Raman and Ramasamy 2017). Since T4HN is an early precursor of the DHN-melanin pathway, the presence of a functional Ayg1 equivalent hydrolase in any *Aspergillus* species is a prerequisite for channeling YWA1 into the DHN-melanin

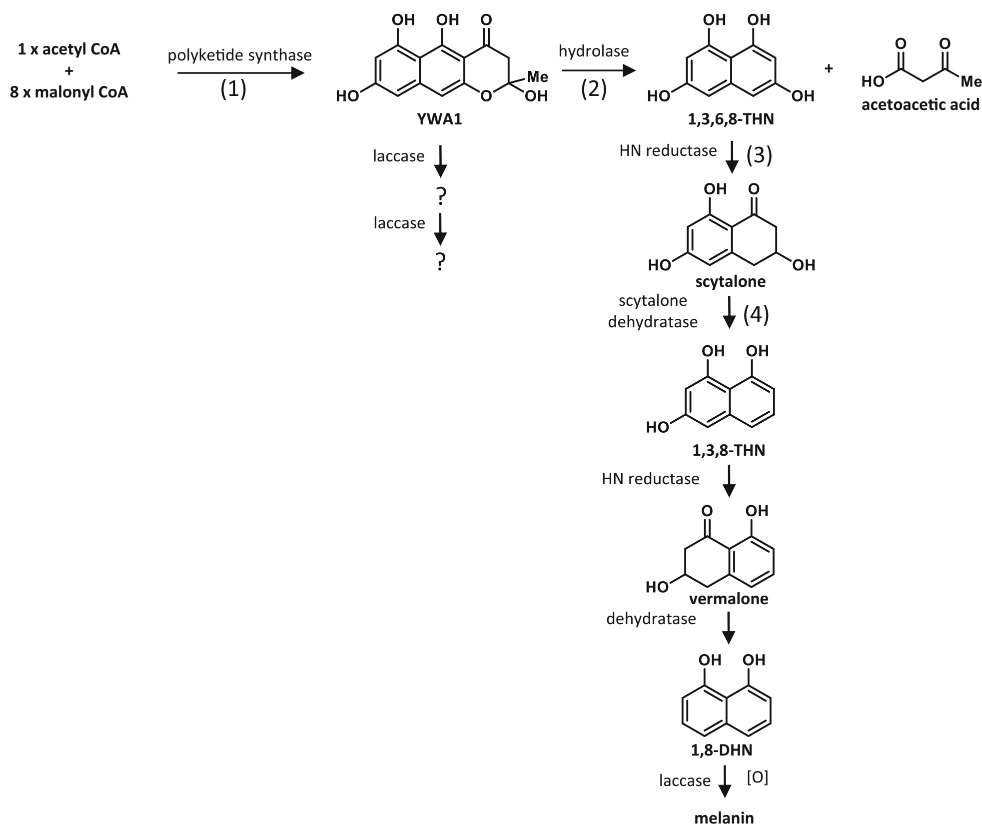

Fig. 1 Formation of an *A. fumigatus* conidial pigment from YWA1 via the 1,8-DHN-melanin pathway. The biosynthetic pathway of the *A. flavus* conidial pigment likely bypasses the shortening step that releases acetoacetic acid from YWA1. The numbers 1, 2, 3, and 4 in the figure correspond to the known *A. fumigatus* genes listed in Table 1

pathway. Gene homologs of *A. fumigatus ayg1* have been identified from a few aspergilli including *A. nidulans*, *A. oryzae*, and *A. niger* (Baker 2008). The *A. niger olvA* knockout mutant produces olive-colored conidia and it can be complemented by An14g05350, an *A. fumigatus ayg1* orthologue, to produce black conidia (Jorgensen et al. 2011). Although this result suggests that the DHN-melanin pathway is operational in *A. niger*, results from other studies argue against such a notion. Despite the fact that *A. niger olvA/ayg1* complements the *ayg1* knockout mutant to wild type, it is not known why the *olvA* knockout mutant produces conidia that are different in color from those produced by the *A. fumigatus ayg1* knockout mutant. Chiang et al. (2011) instead reported that *A. niger aygA/ayg1* knockout mutants produce orange pigmented conidia. The supposed AygA hydrolase of *A. niger* thus is unlikely to perform the "shortening" function as reported for *A. fumigatus* that converts YWA1 to T4HN. In the DHN-melanin pathway, scytalone is an intermediate between T4HN and 1,3,8-THN (T3HN) (Fig. 1). Disruption of the *A. fumigatus arp1* orthologue in *A. niger*, An08g099200, that encodes the scytalone dehydratase, however, does not affect conidial pigmentation of the resulting *A. niger* mutant (Jorgensen et al. 2011). *A. nidulans* produces both conidia and ascospores. The ascospore pigment, ascoquinone A, is a dimer of hydroxyanthraquinone (Brown and Salvo 1994); this metabolite is not related to the DHN-melanin pathway. Taken together, the involvement of the DHN-melanin pathway in the formation of conidial pigments of aspergilli appears to be an exception rather than the rule.

The DHN-melanin pathway has no bearing on *A. flavus* conidial pigment biosynthesis

Homologs of *A. fumigatus ayg1* are present in *A. nidulans* (AN9171) and *A. flavus* (AFLA_075640), but their genuine functions in respective species are not known. For *A. nidulans*, no experimental evidence regarding the function of AN9171 is yet available. For *A. flavus*, disruption of AFLA_075640 does not yield a mutant that is different from the wild type in conidial color (Chang et al. 2010; Saitoh et al. 2012; Tsai et al. 1997). Fungal colonies such as those of *Verticillium dahliae*, *Leptosphaeria maculans*, and *A. fumigatus* that accumulate scytalone are light reddish brown (beige) in appearance (Saitoh et al. 2012; Tsai et al. 1997). Disruption of the *A. flavus* gene (AFLA_016140) encoding scytalone dehydratase, which converts T4HN to T3HN in the DHN-melanin pathway, also does not affect pigmentation of conidia and sclerotia (Cary et al. 2014). These gene knockout results cast doubts on the involvement of the DHN-melanin pathway

in conidial pigment biosynthesis of *A. flavus*. Expression of linked genes tends to be co-regulated similar to those in the *A. fumigatus* conidial pigment biosynthesis gene cluster. Apart from being controlled by promoter sequences and transcription factors, chromosomal locations of the respective *ayg1* orthologues can affect the timing of gene expression, rendering activation of catalytic steps in the conidial pigment biosynthetic pathway differently. The outcome may be the bypassing of the "shortening" route demonstrated for *A. fumigatus*. In addition, melanin pathway inhibitors such as tricyclazole and phthalide, which specifically inhibit reductases that catalyze T4HN and T3HN in the formation of DHN (Chrysayi Tokousbalides and Sisler 1979; Motoyama and Yamaguchi 2003), do not alter *A. flavus* conidial pigmentation (Chang et al. 2019; Wheeler and Klich 1995). Taken together, these findings indicate that the DHN-melanin pathway even if it is intact in *A. flavus* has no bearing on its conidial pigment biosynthesis.

More than one pigment is likely associated with *A. niger* conidial color formation

The black conidial pigment of *A. niger* is aspergillin. Conidia produced by *A. niger* that are treated with 2,4-dithiopyrimide (DTP) accumulate a brown pigment (~ 5000 Da) and a green pigment (~ 368 Da). The latter is thought to be hexahydroxyl pentacyclic quinoid (Ray and Eakin 1975). DTP can chelate intracellular copper ions that are critical for the polymerization function of laccases during conidial pigment biosynthesis. Application of DTP supposedly results in an outcome similar to the *A. flavus* yellow conidial mutant that harbors the defective copper-transporting ATPase gene (Chang et al. 2019). Therefore, aspergillin likely is a polymer consisting of two pigments, that is, the aforementioned brown and green ones or their derivatives. This notion is supported by two lines of evidence: (1) all *A. niger* fawn mutants are complemented by the *pks* gene, An09g05730 (Table 1) (Jorgensen et al. 2011) and (2) the *A. niger albA* knockout mutant does not produce non-pigmented white conidia but instead produces yellowish fawn conidia (Chiang et al. 2011). Like *A. niger*, *A. carbonarius* is another member of the black aspergilli (section *Nigri*). An early study indicates that tricyclazole and other similar inhibitors do not suppress melanin formation in *A. carbonarius* conidia and that its melanin is of the dihydronaphthalene type (Babitskaya et al. 2000). A recent study showed that the *alb1* knockout mutant of *A. carbonarius*, like that of the *A. niger albA* knockout mutant, also produces fawn conidia (Gerin et al. 2018). As mentioned earlier, the *A. niger aygA* knockout mutant produces orange conidia (Chiang et al. 2011). This orange pigment likely reflects the mix of two different precursor pigments, YWA1 and one other yet to be identified and characterized. Thus, conidial pigment biosynthesis in *A. niger* is more complex than previously thought.

The involvement of the DOPA-melanin pathway in some studied aspergilli is questionable

Another type of melanin, which is derived from L-3,4-dihydroxyphenylalanine (L-DOPA) via the oxidation of tyrosine by tyrosinase, is found in some fungi (Fig. 2). This pathway resembles mammalian melanin biosynthesis (Hearing 2011). DOPA-melanin is abundant in two highly melanized *A. nidulans* strains, MEL1 and MEL2 (Goncalves et al. 2012). DOPA-melanin is associated with the chitin fraction distributed throughout the mycelial cell wall of *A. nidulans* (Bull 1970; Pirt and Rowley 1969). However, the involvement of the DOPA-melanin pathway in the formation of conidial pigment of this *Aspergillus* model species is still an open question. Kojic acid and tropolone are inhibitors of the DOPA-melanin pathway. Incorporation of these compounds in growth medium was shown to suppress conidial pigment formation in *A. niger*, *A. flavus*, and *A. tamarii* (Pal et al. 2014). However, the observed inhibitory effect on *A. niger* and *A. flavus* has been confirmed to be caused by the dimethylsulfoxide (DMSO) used to dissolve kojic acid (Chang et al. 2019; Geib and Brock 2017). These findings are consistent with a much earlier study that shows compounds having the sulfoxide radical, including DMSO, inhibit pigmentation of *A. niger* (Carley et al. 1967). Therefore, the conclusion that the conidial pigments of *A. niger* and *A. flavus* are synthesized via the DOPA-melanin pathway is erroneous. Similarly, the association of the DOPA-melanin pathway with *A. tamari* conidial pigment biosynthesis seems inconclusive.

Biosynthesis of conidial pigment in *A. terreus* is unique

Pigment-associated PKSs of *A. nidulans*, *A. fumigatus*, and *A. flavus* (Table 1; *wA*, *alb1*, and *wA*, respectively) share an overall amino acid identity of about 70%. In contrast to known aspergilli, *A. terreus* is an exception as it lacks such a PKS homolog (Thywissen et al. 2011). *A. terreus* instead uses an NRPS-like enzyme (MelA), the only one found so far, and a tyrosinase (TyrP) to synthesize its conidial pigment (Fig. 3). Knockout mutants of *melA* (ATEG_03563) and *tyrP* (ATEG_03564) produce white and bright fluorescent yellow conidia, respectively (Geib et al. 2016). Structure analysis indicates that the bright fluorescent yellow compound is aspulvinone E that originates from condensation of two molecules of p-hydroxyphenylpyruvate (Fig. 3) by MelA. An exogenous addition of tyrosine is able to increase aspulvinone E

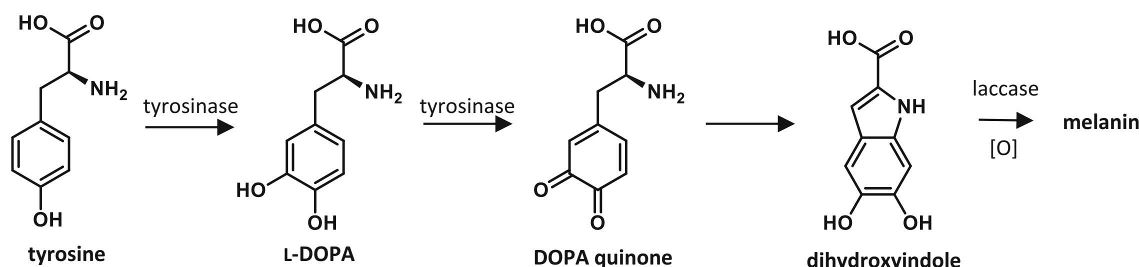

Fig. 2 Tyrosinases form dihydroxyindoles from tyrosine via the L-DOPA pathway

content in a concentration-dependent manner. A blue pigment, that gradually changes to a greenish brown intermediate, appears to form by the activity of TyrP before it is converted to the mature cinnamon-brown pigment, which Geib et al. (2016) named Asp-melanin.

Limited information on fungal sclerotial pigments

In addition to the genus *Aspergillus*, production of sclerotia has been documented among 85 fungal genera in 20 orders of *Dikarya* (i.e., *Basidiomycota* and *Ascomycota*) (Smith et al. 2015). A common feature of most sclerotia is the presence of numerous secondary metabolites, many of which appear to function in chemical defense against insect predators and competing microbes (Rohlfs and Churchill 2011). In *A. flavus*, these include the carcinogenic mycotoxins known as aflatoxins, as well as tremorgenic mycotoxins such as aflatrems and aflavinines (Calvo and Cary 2015). Though a significant amount of attention has been placed on the identification of sclerotial secondary metabolites and their potential as novel pesticides and human therapeutic agents, little emphasis has been placed on the elucidation of metabolites that serve as sclerotial pigments, especially in *Aspergillus* species. These pigments play a part in the long-term viability and persistence of sclerotia in the field by providing protection from UV irradiation and extreme temperature and resistance to fungivory and microbial degradation (Liang et al. 2018; Rohlfs and Churchill 2011; Schumacher 2016). In general, pigments present in sclerotia are associated with the outer rind layer of the mature sclerotium (Willetts and Bullock 1992). The majority of studies on sclerotial pigments have been performed in the necrotrophic fungal pathogen, *Sclerotinia sclerotiorum*. The dark pigments present in *S. sclerotiorum* are DHN-melanins derived from the activity of a PKS (Butler et al. 2009). Interestingly, knockout of the DHN-melanin biosynthetic genes, *SCD1* and *THR1*, does not completely abolish sclerotial pigmentation in *S. sclerotiorum*, indicating that perhaps an alternative melanin biosynthetic pathway is functional (Liang et al. 2018). It has also been confirmed that DHN-melanin-based pigments are present in sclerotia of the causal agent of gray mold disease, *Botrytis cinerea* (Schumacher 2016). However, two separate PKSs both capable of producing the DHN-melanin precursors, T4HN and 2-acetyl-tetrahydroxynaphthalene (AT4HN), are present. One PKS, BcPKS12, has been demonstrated to be required for T4HN production in sclerotia only, while BcPKS13 is responsible for AT4HN production in conidia that is subsequently converted to T4HN by the action of the hydrolase YG1. Numerous *Aspergillus* species produce sclerotia whose pigments are highly variable, ranging from cream colored to reddish brown to dark brown or black (Frisvad et al. 2004; Frisvad et al. 2019; Frisvad et al. 2014). *A. flavus* produces immature sclerotia that are essentially colorless and as they mature become progressively darker until they reach a dark brown to black pigmentation at final maturity. To date, very little information exists as to the chemical nature of

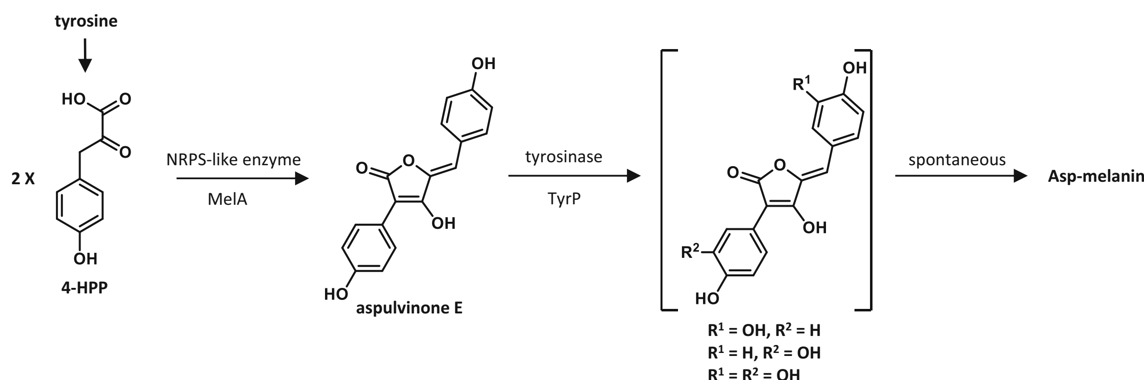

Fig. 3 Formation of Asp-melanin from tyrosine via an NRPS-type enzyme. MelA produces aspulvinone E, which is oxidized by TyrP to intermediates that can spontaneous form Asp-melanin

sclerotial pigments in aspergilli. The existence of paler colored sclerotial pigments, such as those found in species of *Aspergillus* section *Circumdati* (Frisvad et al. 2004), suggests that these pigments are not derived from the DHN-melanin pathway.

Identification of the *A. flavus* sclerotium-specific pigment precursor asparasone A

A comparative transcriptomic analysis of a wild type *A. flavus* and its *veA* gene knockout mutant has shown that a PKS gene, present in the secondary metabolite gene cluster 27, is significantly downregulated in the mutant (Cary et al. 2014). Knockout of the cluster 27 PKS gene (*pks27*, AFLA_082150) yields a mutant that no longer produces darkly pigmented but grayish-yellow-pigmented sclerotia. Comparative metabolomics of culture extracts from both the *A. flavus* wild type and the *pks27* knockout mutant by UHPLC-MS revealed a metabolite of mass 358 Da that was identified as the anthraquinone, asparasone A. Also detected was the dehydration product of asparsone A (mass = 340 Da) as well as another anthraquinone (mass = 316 Da), believed to represent a derailment product in which only seven malonyl-CoA units are used to form the polyketide instead of the eight present in asparasone A (Fig. 4). It has been theorized that subsequent dehydration of asparasone A, or the 316 Da anthraquinone, would result in conjugated olefins that are rapidly polymerized in the presence of laccases to form the dark pigments' characteristic of *A. flavus* sclerotia (Cary et al. 2014). These studies indicate that unlike the use of polyketide-derived naphthoquinone precursors in the production of DHN-melanins in sclerotia of *S. sclerotiorum* and *B. cinerea*, *A. flavus* sclerotial pigments are formed from anthraquinone precursors. This proposition is further supported by the observation that *A. flavus* DHN-melanin biosynthetic pathway scytalone dehydratase gene knockout mutants do not show reduced sclerotial pigmentation compared with sclerotia of wild type *A. flavus* (Cary et al. 2014). The gene cluster responsible for the production of asparasone A appears to be present only in section *Flavi Aspergillus* species, suggesting that the cluster evolved in response to ecological pressures linked to the need for these fungi to survive and successfully reproduce in hostile agrarian environments. The *aswA* transcription factor gene that regulates sclerotial development in *A. flavus* has been identified and functionally characterized (Chang et al. 2017). Knockout of the *aswA* gene results in mutants that produce non-pigmented sclerotia. The production of these sclerotia in *aswA* knockout mutants and that of grayish-yellow pigmented sclerotia in the *pks27* knockout mutants suggests that an additional pigment(s), whose synthesis may be regulated by *aswA*, might be present but masked by the darker asparasone A-derived pigment in mature sclerotia.

Transport and localization of conidial pigments to cell wall

In mammals, DOPA-melanin is synthesized in a lysosome-related organelle (LRO) known as the melanosome by specialized cells called melanocytes. In them, melanin is synthesized and deposited onto the fibrillary matrix and the resulting melanosomes are transferred to neighboring keratinocytes by exocytosis and internalization (Kondo and Hearing 2011). Fungi appear to share similar mechanisms for synthesis and trafficking of conidial pigments. Internal melanosome-like organelles have been reported for *Candida albicans* (Walker et al. 2010) and *Cladosporium carrionii* (San-Blas et al. 1996). *Fonsecaea pedrosoi*, a human pathogenic fungus, produces dark-brown conidia. Its conidial pigment is synthesized via the DHN-melanin pathway since treatment by tricyclazole inhibits pigmentation of conidia and sclerotia as well (Franzen et al. 2006). Ultrastructural characterization has revealed that the *F. pedrosoi* melanosome fuses with cell membrane, and subsequently, the DHN-melanin derived pigment is released and deposited on the conidial cell wall in concentric layers (Franzen et al. 2008). The finding of *A. terreus* TyrP, which hydroxylates and oxidizes aspulvinone E, in subcellular organelles like endoplasmic reticulum or Golgi (Geib et al. 2016)

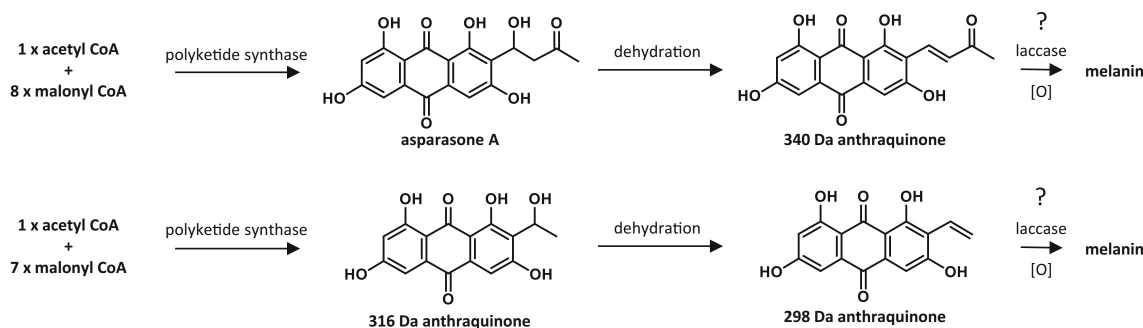

Fig. 4 Proposed biosynthetic routes for the formation of an *A. flavus* sclerotial pigment from two polyketides

also suggests that the resulting conidial pigment is probably transported via a similar exocytosis mechanism. For *A. fumigatus* and *A. nidulans*, enzymes involved in early steps of conidial pigment biosynthesis are located in LROs called endosomes (Upadhyay et al. 2016). A defect in the endosomal sorting complex in these aspergilli results in the lack of mature pigment in conidial cell wall. Interestingly, late biosynthetic enzymes for pigment formation are found to be secreted and accumulate in conidial cell wall. This stage-specific subcellular compartmentalization is supposedly designed for protecting cells from harmful effects of those melanin-like pigments and their intermediates, which presumably are highly reactive and tend to bind inter- and intracellular substances on contact.

Concluding remarks

Despite decades of efforts, research on chemical structures and biosynthetic pathways of conidial and sclerotial pigments in aspergilli is still at its infancy. Isolation and identification of the YWA1 monomer naphthopyrone were achieved a decade ago. This was possible only because of the use of a heterologous over-expression system that expresses the *A. nidulans wA* gene in a YWA1 non-producing *A. oryzae* strain. Since then, with exception of the anthraquinone, asparasone A, that is isolated from the sclerotia of *A. flavus* and Asp-melanin isolated from conidia of *A. terreus*, virtually no other conidial or sclerotial pigments of aspergilli have been characterized. Coupled transcriptomic and metabolomic analysis of *A. flavus* conidial and sclerotial mutants should provide additional clues as to the genes and enzymes responsible for production of pigments in these fungal structures. Detailed analysis of melanin-type pigments using current analytical methodologies has proved difficult because of the heterogeneity and insolubility of these amorphous polymers over a wide range of pH and solvents. Treating melanin with harsh chemicals (Nosanchuk et al. 2015) or using non-destructive methods like solid-state nuclear magnetic resonance with isotopic labeling may be alternatives (Chatterjee et al. 2014). The significance of the DHN- and DOPA-melanin pathways in the biosynthesis of these pigments is still unclear and controversial. This brief review identifies main research gaps, suggests future research avenues, and points out challenges ahead in elucidating the formation of these fascinating and enigmatic pigments at the molecular level.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain studies with human participants or animals.

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