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Azaphilones are fungal polyketide pigments bearing a highly oxygenated pyranoquinone bicyclic core which receive an increasing great deal of research interest for their applications on agroalimentary, dyeing, cosmetic, printing and pharmacy industries. Their biosynthetic pathways are not fully elucidated but thanks to recent genomic approaches combined to the increasing genome sequencing of fungi some of them have been recently unveiled. Here is the first review on the biosynthesis of azaphilonoids adressed from a genomic point of view.

Introduction

Azaphilone compounds are fungal polyketides produced by numerous species of ascomyceteous and basidiomyceteous fungi. Besides their well-known pigment functions, azaphilones exhibit a wide range of potent biological activities. Many of these activities are attributed to their affinity for the amino groups present in proteins or nucleic acids. In view of these promising biological activities, pigment functions and interesting structural features, azaphilones have increasingly received a great deal of research interest. Earlier studies of azaphilone biosynthesis, using isotope labeled substrates, allowed an outline of the pathway to be proposed. Subsequently, genomic approaches combined to the increasing genome sequencing of fungi allowed discovering the biosynthetic gene clusters (BGCs) involved in the azaphilones biosynthesis, and paved the way to deciphering it. In this highlight, we thus review the recent findings regarding the biosynthetic gene clusters involved in the production of azaphilones.

Results

Azaphilones are produced by 61 genera of fungi belonging to *Annulohypoxylon spp., Ascochyta spp., Aspergillus spp., Bartalinia sp., Beauveria sp., Biscogniauxia sp., Bulgaria spp., Cephalotheca sp., Cercosporella sp., Chaetomium spp., Cladosporium sp., Cochliobolus spp., Colletotrichum spp., Coniella sp., Creosphaeria sp., Curvularia sp., Cyathus spp., Cylindrocarpon spp., Cytospora sp., Daldinia spp., Delitschia spp., Diaporthe sp., Dothideomycete sp., Emericella spp., Entonaema sp., Epicoccum nigrum, Eupenicillium sp., Fusarium spp., Fusidium sp., Glaziella sp., Helotialean spp., Hypocrella sp., Hypoxylon spp., Leptosphaeria spp., Microdochium sp., Monascus spp., Montagnulaceae sp., Mycoleptodiscus sp., Nemania sp., Nidularia sp., Nigrospora sp., Penicillium spp., Pestalotiopsis sp., Peyronellaea sp., Phialophora sp., Phomopsis spp., Pithomyces spp., Pleosporales sp. Pleurostomophora sp., Pochonia sp., Podospora sp., Pseudohalonectria spp., Sepedonium sp., Stachybotrys sp., Talaromyces spp., Thielavia sp., Trichocladium sp. Trichoderma sp., Trichopezizella sp., Xylariales sp. and Zopfiella sp.*. From all those genera 677 azaphilones have already been described and a substantial updated azaphilone database is provided in Table S1 describing the name of the azaphilone along with its structural chemical family, the producing strains, its molecular formula and neutral mass. This work will prove to be of significance for fungal dereplication processes in the azaphilone series.

This review is intended to continue the coverage of the literature previously reviewed in this journal by Chen W. (1) and recently by Chen C (2). It will mainly focus on the recent biosynthetic studies of azaphilones for which the biosynthetic genes/enzymes have been identified. Five main biosynthetic pathways have been described among the 61 genera of fungi, so far: The *Monascus* azaphilone pathway (MAzPs), the *Aspergillus* azaphilone pathway, the citrinin pathway, the *Chaetomium*/cochliodone pathway as well as the *Hypoxylon* pathway.

The five described azaphilone biosynthetic pathways share a common beginning. Either a non-reducing PKS (nrPKS), or a highly-reducing (hrPKS) and a nrPKS, build a common orcinaldehyde intermediate which can be either tailored by enzymes towards the citrinin pathway or hydroxylated by a monooxygenase to produce a common pyranoquinone core tailored to the MazPs, *Aspergillus* azaphilone pathway, *Chaetomium*/cochliodone or *Hypoxylon* pathway (see Figure 1).

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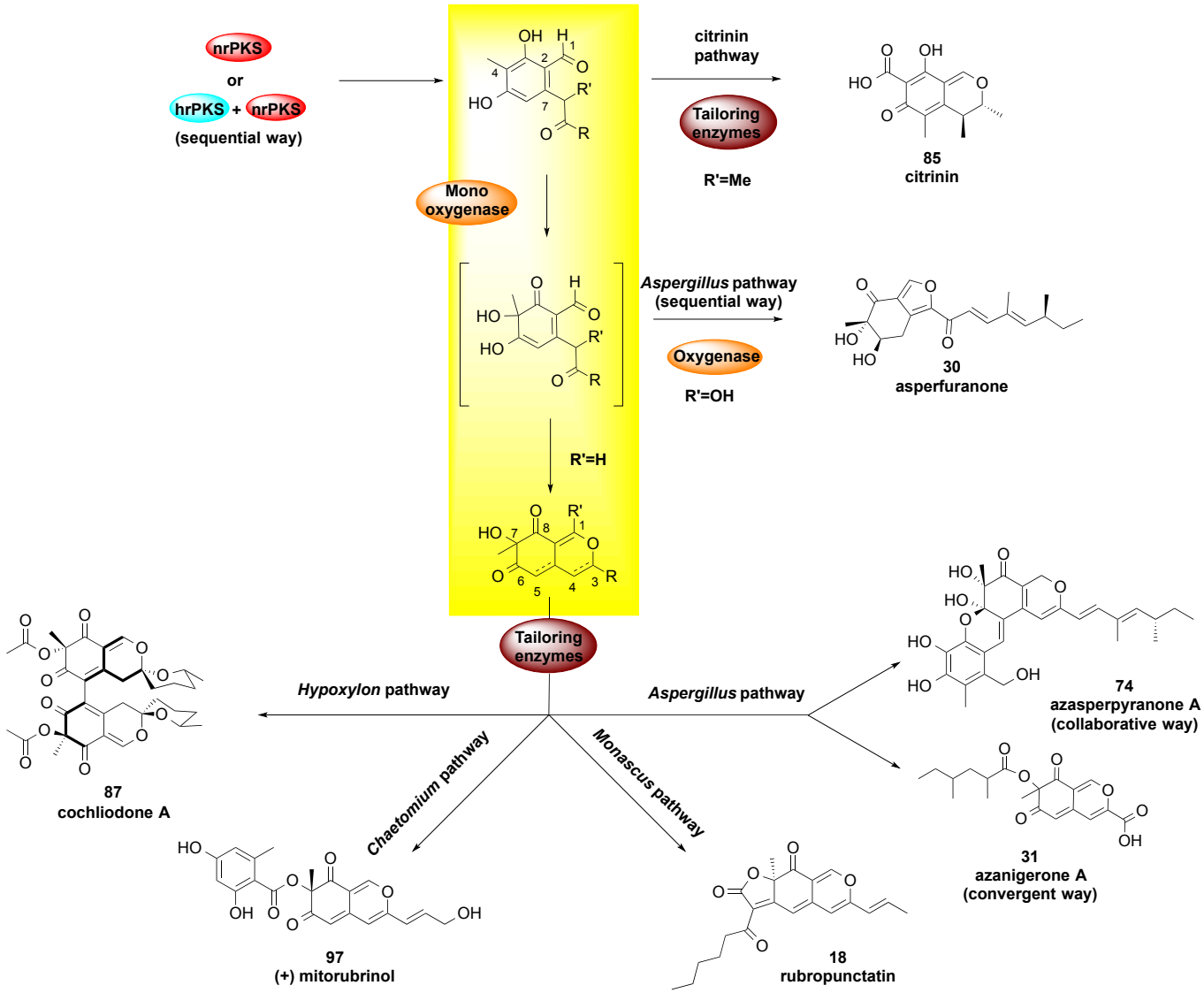

Figure 1 Described azaphilones biosynthetic pathways

I. The *Monascus* azaphilone pathway

Seven MAzPS biosynthetic gene clusters were described from the *Monascus* genus: *M. purpureus M3103* (3)*, M. purpureus NRRL 1596, M. purpureus YY-1, M. pilosus, M. ruber M7,*(4) *M. purpureus KACC42430* and *M. ruber NRRL 1597* and four on the *Talaromyces* genera: *Talaromyces aculeatus ATCC10409, T. atroroseus IBT11181, T. marneffei ATCC18224* and *T. stipitatus ATCC10500* (1). The comparison of the clusters revealed that the genes are conserved but the physical order of the genes is different between *Monascus* and *Talaromyces.* Thanks to the efforts of few teams (4–14) using a combination of gene knockouts, heterologous expression and *in vitro* reconstitution of selected reactions, knowledge concerning the biosynthesis pathway of *Monascus* azaphilone pigments was considerably improved (Figure 2).

The MonAzPs biosynthesis is initiated by a nrPKS (MrPigA in *M. ruber* or MpPKS5 in *M. purpureus*) featuring eight domains as follow: a starting unit acyltransferase (SAT), a keto-synthase (KS), an acyltransferase (AT), a product template (PT), a C-methyltransferase (MT), two acyl carrier proteins (ACP), and a reductive release domain (R). Briefly, according to Chen *et al.* (4) the SAT domain of MrPigA selects an acetyl-CoA starter unit which is extended five

times with malonyl-CoA in successive decarboxylative Claisen condensation cycles thanks to KS, AT and ACP domains. Then, the methyltransferase (MT) domain of MrPigA conducts a single C-methylation at C-4, most likely at the pentaketide stage. The reactive hexaketide chain then undergoes a product template (PT) domain-mediated C-2 to C-7 aldol cyclization to afford the aromatic ring followed by reductive release catalysed by the R domain of MrPigA to afford the putative benzaldehyde intermediate **2**. A deduced serine hydrolase MrPigG may help the release of the reactive intermediate **2** from MrPigA (4).

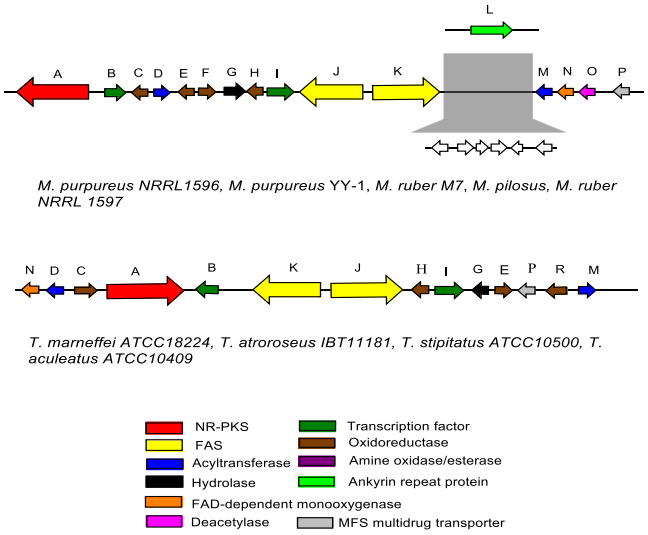

Figure 2 MonAzPs biosynthetic gene clusters for *Monascus* and *Talaromyces* genera (1)

It should be noted that according to Balakrishnan *et al.* MrPigA contained only one acyl-carrier protein while Chen *et al.* described two ACP (5). To the best of our knowledge, the importance or interest of the second ACP in MrPigA has yet to be discussed.

The MrPigC therefore intercepts the reactive benzaldehyde **2** to reduce the ω-1 carbonyl into the corresponding alcohol **3**. Interestingly, it has been found that, in *M. ruber* M7, in case of deletion, other enzyme(s) can carry out the same C-11 reduction (15). The alcohol **3** is then oxidized by MrPigN (or MppF), a FAD-dependent monooxygenase, allowing the formation of the bicyclic pyranoquinone skeleton **4**. On the other hand, MrPigJ ((or MpFasA2) a FAS subunit alpha) and MrPigK ((or MpFasB2) a FAS subunit beta) produce the side chain fatty acyl moiety of MonAzPs. The β-ketoacyl-ACP probably serves as the substrate for the AT MrPigD (or MppB) that directly transfers the fatty acyl chain to the C-4 alcohol of (**4**) to produce the putative intermediate (**5**).

To produce the yellow pigments monasfluol A (**7**) and B (**8**), the pyranoquinone **5** is subjected to intramolecular Knoevenagel aldol condensations, leading to a tricyclic ring system **6** that is possibly reduced by MrPigH (or MppE), an enoyl reductase.

The previously described pyranoquinone **5** could also undergo a two-step acetylation-elimination sequence successively catalyzed by MrPigM (or Mpp7), an *O*-acetyltransferase, and MrPigO (or Mpp8), a deacetylase leading to the putative unsaturated intermediate **10**. This latter may therefore go through two paths. One involves a regioselective Knoevenagel reaction, introducing the angular lactone, and a putative reduction by MrPigH to produce the yellow pigments monasfluore A and B (**12** and **13**).
The other path involves at first MrPigE (or MppC) a NAD(P)H-dependent oxidoreductase which reduces the C4a(5) double bond of **10** followed by a regioselective Knoevenagel reaction, introducing the linear lactone and creating the tricyclic ring system **15.** It should be noted that either linear or angular lactones could be obtained by Knoevenagel reaction, depending on the electronic nature of the π-conjugated system of the *4H*-pyran-4-ylidene moiety (8). In addition, it has also been reported that enzymatically controlled Knoevenagel reaction (Mpp7) could lead to the linear lactone whereas spontaneous condensation gave the angular one (14). Reduction of the furanone moiety of the linear tricyclic products may lead to the yellow pigments monascin (**16**) and ankaflavin (**17**) while its C4a(5) double bond can be oxidized by MrPigF ((or MppG) a FAD-dependent oxidoreductase) to afford the orange pigments rubropunctatin (**18**) and monascorubrin (**19**). These two orange pigments can also lead to the formation of the red pigments rubropunctamine (**20**) and monascorubramine (**21**) by a direct reaction with endogenous ammonia or amino-acids (Figure 2).

The MAzPS cluster also contains several others genes like MrPigI (or MppR2) a transcription factor whose role in the biosynthesis pathway is unknown or MrPigB (or MppR1) a transcription factor known to control the expression of MrPigA, MrPigH, MrPigM, MrPigO, MrPigN and MrPigP (15). MrPigL (or Mpp15) is a ankyrin repeat protein but there is no functional verification so far (1). MrPigP (or MppI) annotated as a MFS multidrug transporter was found not to be involved in the biosynthesis or the export of the *Monascus* azaphilones (15).

II. The *Aspergillus* azaphilone pathway

The biosynthetic pathway of azaphilones involving an hrPKS-nrPKS action in tandem was primarily found in the *Aspergillus* genus and has been described in *Aspergillus nidulans* (16)*, Chaetomium globosum* (17)*, Ascomycete sp.* F53(18)*, Aspergillus terreus* (19) and *Aspergillus niger* ATCC 1015 (20).

The nrPKS and hrPKS can act in sequential, convergent, mixed or collaborative ways. In the sequential way, the first PKS forms a polyketide chain, which is later transferred to the second PKS to continue its elongation process. This case has been found in the asperfuranone biosynthesis (16). In the convergent way, the nrPKS and hrPKS can both function independently in parallel, and the two polyketide products are connected by accessory enzymes. This way is involved in the biosynthesis of azanigerone (20). In *Chaetomium globosum*, the *caz* biosynthetic cluster harbors a hrPKS acting both in a sequential and convergent manner with a nrPKS to produce the chaetoviridins or the chaetomugilin A. Finally, azasperpyranone is produced by a collaborative way involving two separate BGCs coordinated by transcriptional crosstalk (19).

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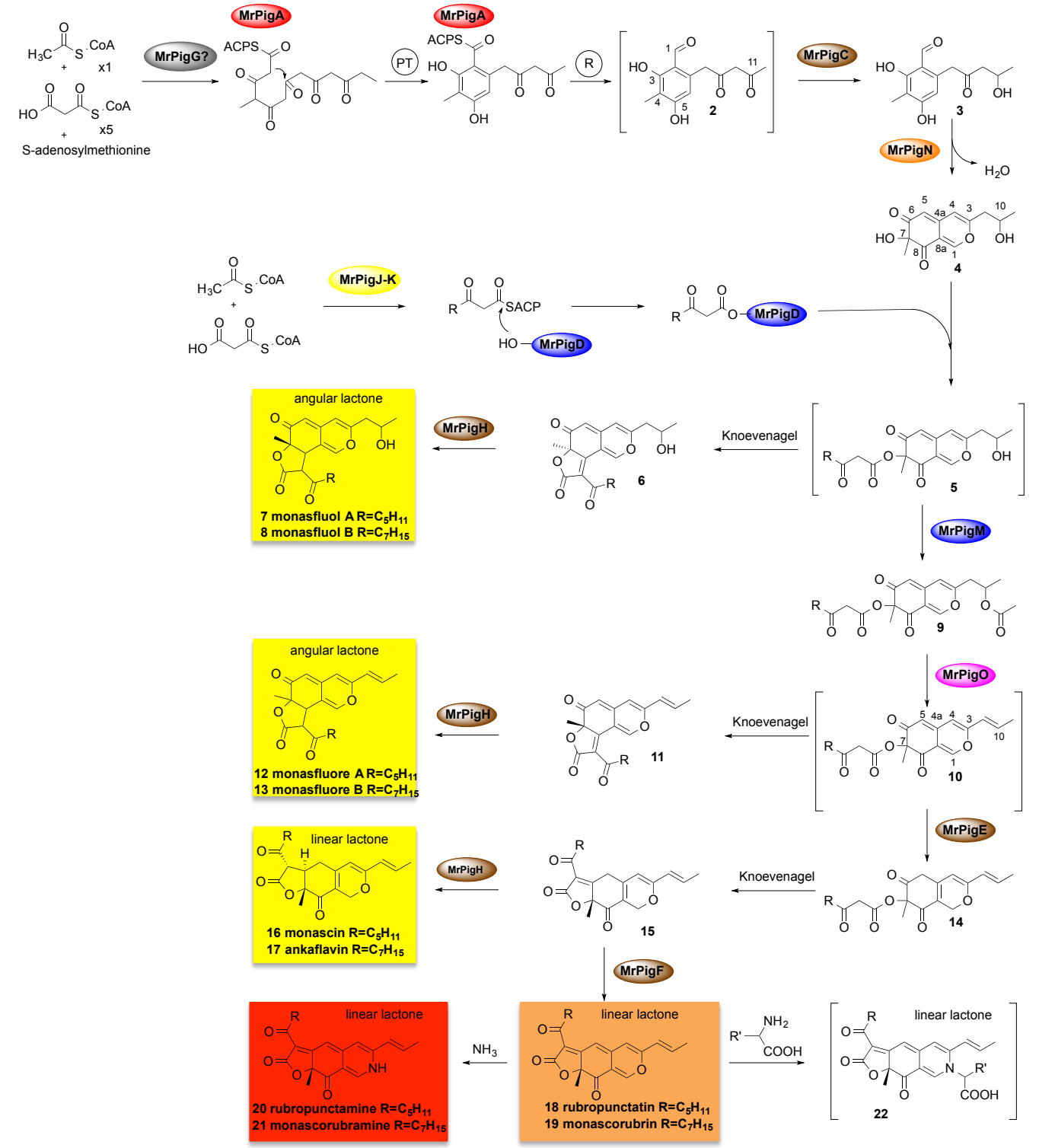

Figure 3 Proposed biosynthetic pathway for MonAzPs in *M. ruber* M7 (4)

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a. Sequential way

Chiang *et al.* described in *A. nidulans* a cryptic gene cluster under normal laboratory growth conditions containing a nrPKS near a hrPKS (16). Closer examination of the gene cluster revealed that AfoA (coded by AN1029.3 gene) shares a high homology with CtnR, a citrinin biosynthesis transcriptional activator. By inducing the transcription activator located next to the two PKSs genes, Chiang *et al.* activated the PKS cryptic pathway involved in the asperfuranone biosynthesis. A series of gene deletion experiments allowed proposing the asperfuranone biosynthetic pathway that includes two PKSs (A*foE* for the nrPKS and AfoG for the hrPKS) together with three additional proteinsAfoC, AfoF hydroxlating C-7 and AfoD hypothesized to hydroxylate C-7 which, in fact, hydroxylates C-3 .(21,22)

Domain composition of AfoE was described as follow : SAT/KS/AT/PT/ACP/CMeT/R, when AfoG contained the domains : KS/AT/DH/CMeT/ER/KR/ACP.

Later, the putative asperfuranone gene cluster from *A. terreus* (*ateafoA-G)*, highly homologous with the *afoA-G* gene cluster was cloned. By using *A. nidulans* heterologous expression system, they clarified the asperfuranone biosynthetic pathway (23).

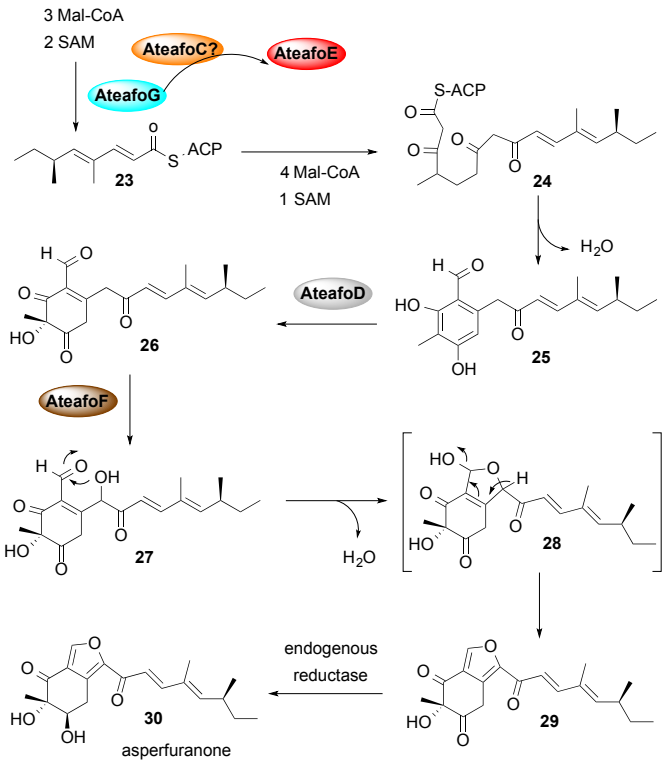

Figure 4 Proposed biosynthesis pathway for asperfuranone in *A. nidulans* (16, 21)

The hrPKS AteafoG is responsible for the production of 3,5-dimethyloctadienone, which is loaded onto the SAT domain of the nrPKS AteafoE for further extensions. Their results suggest that AteafoC might be involved in the releasing of **23** from AteAfoG or might facilitate the transfer from AteAfoG to AteAfoE. Then, the nrPKS AteAfoE uses four Malonyl-CoA and one SAM to produce the pentaone **24**, which undergoes a cyclization to afford the aromatic ring **25**. AteAfoD is proposed to have a dearomatization function of the precursor **25** to generate the hydroxylated intermediate **26**. AteAfoF functions as a C-8 oxygenase to generate **27** which then undergoes spontaneous cyclization leading to compound **29** which is converted in asperfuranone (**30**) by an endogenous reductase (Figure 4).

b. Convergent way

In 2012, Zabala *et al.* found a similar cryptic gene cluster(20), using a convergent mode of collaboration for the hrPKS and nrPKS, on *Aspergillus niger ATCC 1015* and compared it to the one previously found in *Aspergillus nidulans* (Figure 5).

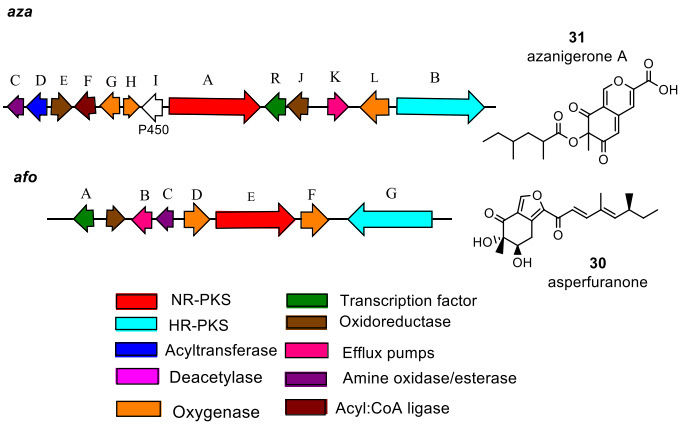

Figure 5 Comparison of the *A. niger* Aza cluster with the *A. nidulans* Afo cluster and their respective products. (20)

The biosynthetic pathway of asperfuranone (**30**) and azanigerone A (**31**) share numerous homologs. Indeed, the AzaC, AzaG, AzaH, AzaJ and AzaL are tailoring enzymes (homologs of AfoC, AfoF, AfoD, AN1030 and AfoF respectively). It is noteworthy that function of AzaC remains enigmatic, the AzaB hrPKS (AfoG homolog) producing the dimethylhexanoate **38**, the AzaA nrPKS (AfoE homolog) giving the aromatic compound **34**, and the AzaR being a transcription factor (AfoA homolog). The main differences between asperfuranone and azanigerone pathways lie in the involvement for this latter of the AzaD acyltransferase in the transfer of the dimethylhexanoate **38** on the pyranoquinone **36** to produce the azaphilone **41**, the AzaE ketoreductase which reduces the ketone

function of **34** into hydroxyl, the AzaF AMP-dependent CoA ligase responsible of the ligation of **38** and the AzaI cytochrome P450 which oxidizes the hydroxyl of **42** into an aldehyde (Figure 6). Interestingly, Zabala *et al.* found that AzaH, a key FAD-dependent monooxygenase encoded in the aza cluster had a role in the stereoselective (24,25) hydroxylation at C-4 promoting thus the pyran-ring formation and leading to the characteristic bicyclic core of azaphilones (20).

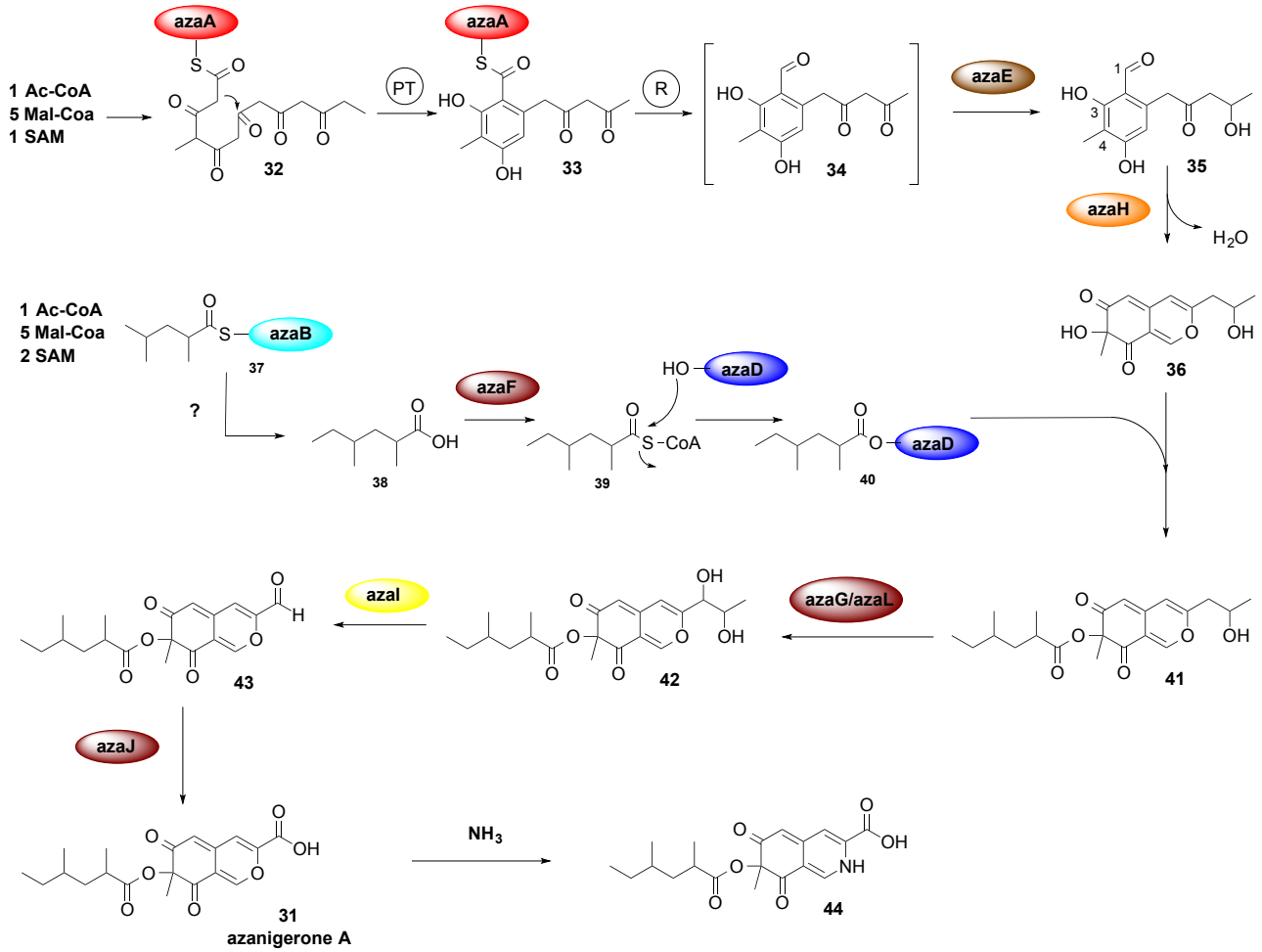

Figure 6 Proposed biosynthetic pathway for production of azanigerones. (20)

The aza pathway represented the first example of a convergent mode of collaboration between a hr- and a nrPKS, as opposed to the sequential mode of collaboration commonly observed in other dual PKS systems. Then, in March 2020, Jesse W. Cain *et al.* described the new azaphilone, lijiquinone, isolated from *Ascomycete sp. F53* which is likely to be biosynthesized via a convergent pathway related to the one of *Aspergillus* (18).

c. Sequential and convergent ways in *Chaetomium globosum*

In *Chaetomium globosum*, the nrPKS (CazM) and the hrPKS (CazF) were described to act in both a sequential and convergent fashion (17) leading to chaetoviridin A (**53**) and chaetomugilin A (**56**).

The Caz biosynthetic gene cluster contains the CazM possessing domains described as follows : SAT/KS/MAT/PT/MT/ACP/R and the CazF possessing : KS/MAT/DH/MT/ER/KR/ACP (17,26).

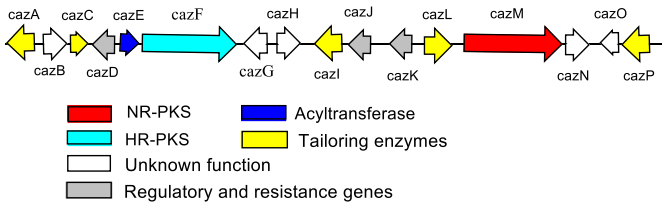

Figure 7 Caz biosynthetic gene cluster in *C. globosum* (17)

The hrPKS CazF most likely provides a highly reduced triketide **45** specifically selected by the SAT domain of the nrPKS CazM as start unit to produce the aromatic compound **47** in a collaborative mode of interaction between the two PKSs. This latter will be then processed by the halogenase CazI followed by a hydroxylation-catalyzed annulation by the predictive monooyygenase CazL to afford the chloro-pyranoquinone named cazisochromene (**49**) (27). The additional oxidized triketide **50** produced by CazF is then added

by the acyltransferase CazE to the pyranoquinone **49** giving the compound **52**. The pyranoquinone **52** after Knoevenagel reaction leads to the tricyclic angular lactone **53**, namely chaetoviridin A which relative side-chain configuration was revised recently (28). Chaetoviridin A (**53**) then evolves to other chaetoviridins and chaetomugilins by rearrangements, reductions, oxidations or reaction with amines (Figure 8).

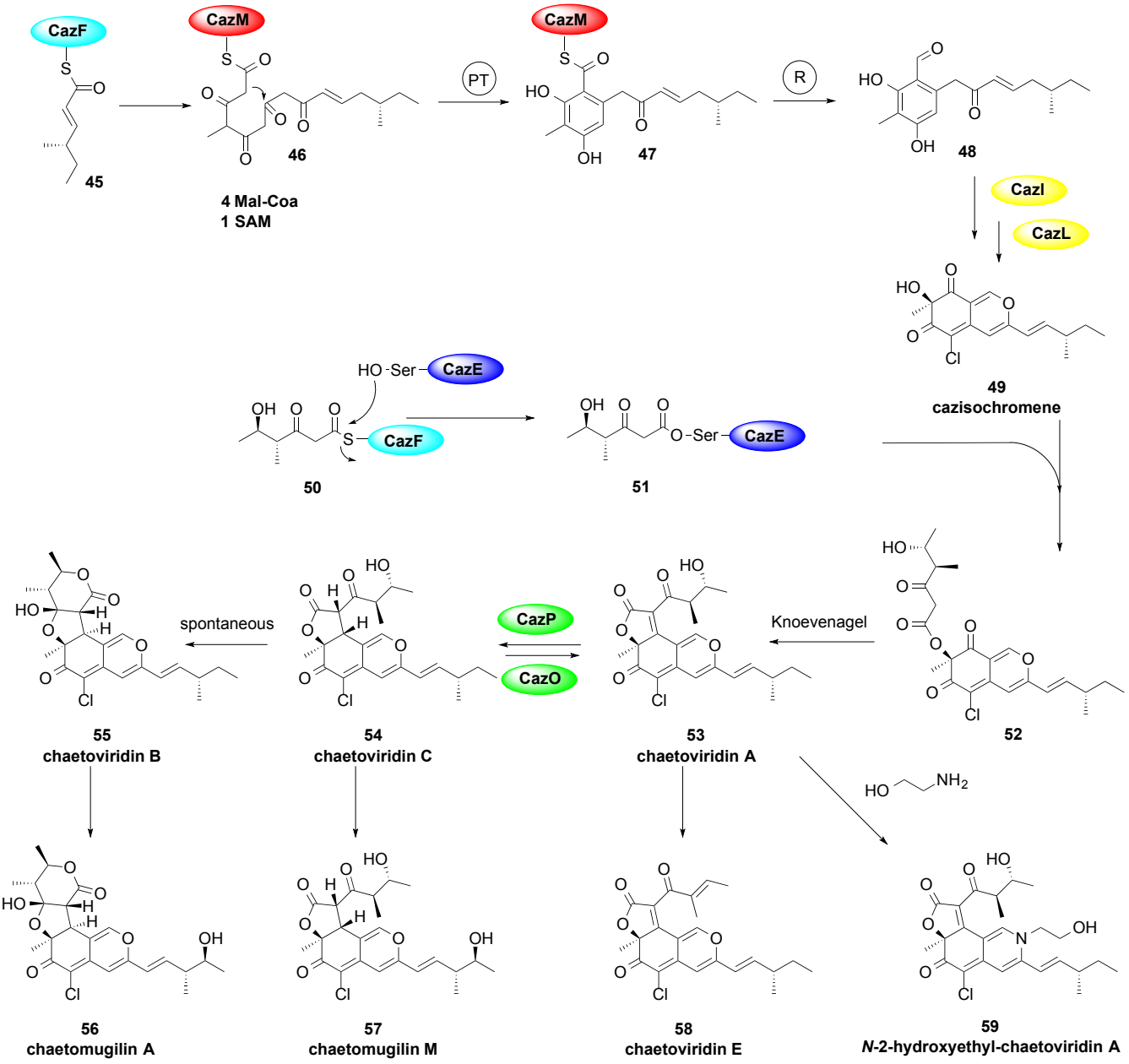

Figure 8 Proposed biosynthetic pathway of chaetoviridins and chaetomugilins (17, 23)

d. Collaborative biosynthesis

In the matter of the mode of collaboration between polyketide synthases, Huang *et al.* provided further data in 2020 (19). Indeed transcriptome analysis of *A. terreus* showed that the production of the azaphilone azasperpyranone A is correlated to the strongly co-transcription of two pairs of core megasynthase genes located at two separate BGCs which have been previously, separately characterized through heterologous expression, because both of them were silent in native *A. terreus* strains. Gene deletions and biochemical investigations demonstrated that the azaphilone azasperpyranone A and derivatives were collaboratively synthetized by two separate clusters containing four core-enzymes, two nrPKSs, one hrPKS and one NRPS-like. The biosynthesis was found to be coordinately regulated by a transcriptional crosstalk between the two gene clusters involving three transcriptional factors.

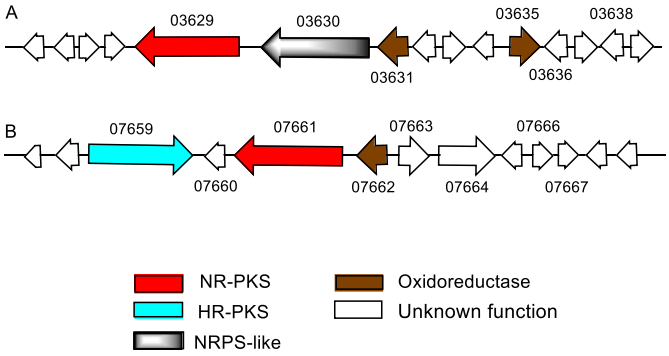

Figure 9 Genetic organization of the two BGCs (19)

The cluster A includes ATEG_03629 and ATEG_03630 encoding nrPKS and a NRPS-like respectively and is responsible of the polyhydric phenol moiety while cluster B includes ATEG_07659 and ATEG_07661 encoding an hrPKS and a nrPKS, respectively led to the azaphilonoid scaffold **21** (see Figure 10).

Briefly, hrPKS ATEG_07659 gives a reduced polyketide elongated by the nrPKS ATEG_07661 and hydroxylated in C-4 by a FAD-dependent oxygenase ATEG_07662 to afford an aromatic ring intermediate leading to the the preasperpyranone (**64**). On the other hand, nrPKS ATEG_03629 produces orsellinic acid (**67**) which is then reduced by the NRPS-like ATEG_03630 leading to the benzaldehyde **69**. Hydroxylations are thus performed by ATEG_03635 and ATEG_03631 leading to the unstable compound **71** which most likely conducts to the stable lactone **73**. Enzymatic mechanisms involved in the covalent bond formation between the two scaffolds **64** and **71** leading to azasperpyranone A (**74**) still remain unknown.

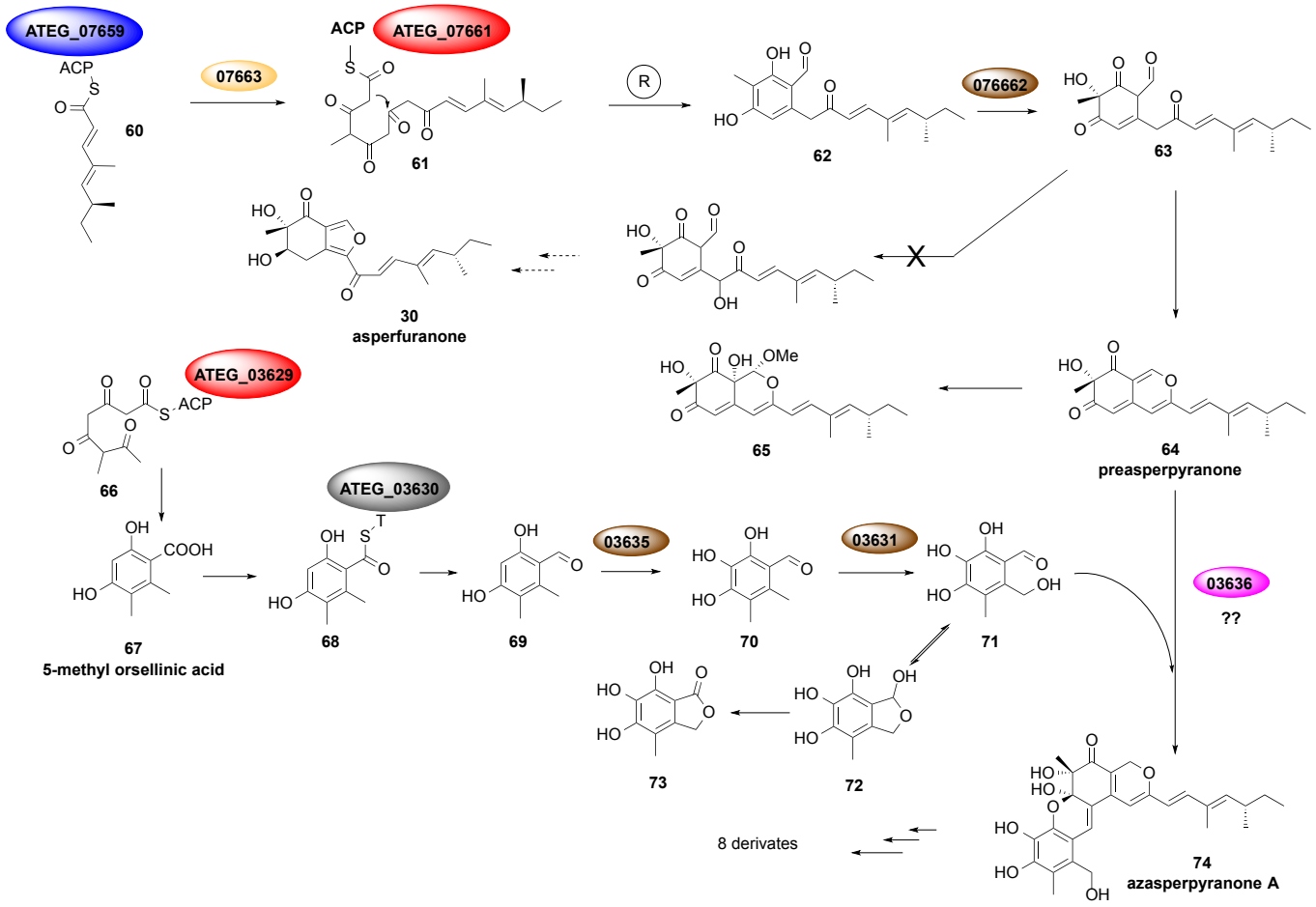

Figure 10 Proposed biosynthetic pathway of asperufranone and azasperpyranone A (19)

III. The citrinin pathway

Citrinin (**85**) is a polyketide-derived mycotoxin first isolated from *Penicillium citrinum* by Hetherington and Raistrick in 1931 and later from other *Penicillium species* as well as *Aspergillus* and *Monascus species* including *Monascus ruber* and *Monascus purpureus* (29,30).

In 2015, little was still known of the individual chemical assembly steps of citrinin biosynthesis before He and Cox led the way, using targeted gene knockout and heterologous expression in *Aspergillus oryzae* with strong promoters to determine the steps of the citrinin biosynthesis in *Monascus ruber M7* (30).

They found that the biosynthetic pathway involved CitS, a nrPKS with the domains SAT/KS/AT/PT/ACP/C-MeT/R, assisted by the hydrolase CitA, which was previously wrongly annotated as an

oxidase, as well as oxidoreductases and hydrolases (named CitB, CitC, CitD, CitE; Figure 10). Briefly, they demonstrated that CitB is a non-heme iron oxidase, which oxidises the 12-methyl group of the keto-aldehyde **81** to benzyl-alcohol **82**. They highlighted that subsequent steps are catalysed by CitC, which oxidises the benzylic 12-hydroxyl to aldehyde **83** and CitD, which converts the 12-benzaldehyde to the benzoic acid **84**. Final oxidation of C-3 by CitE yields citrinin (**85**).

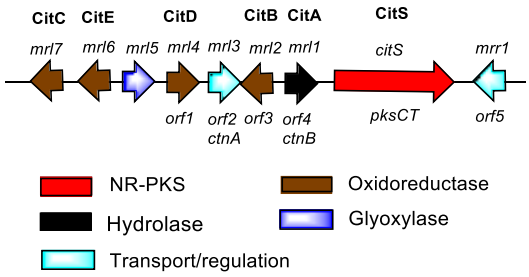

Figure 11 Proposed citrinin gene cluster from *Monascus* species (26)

Storm *et al.* in 2017 shed a new light on the nrPKS PksCT by domain deconstruction and reconstitution and demonstrated the role of CMeT-catalyzed methylation in precursor elongation and pentaketide formation as represented in Figure 11. Indeed, methyl groups added by the CMeT would play the role of check-point tags that are recognized by KS domain (31).

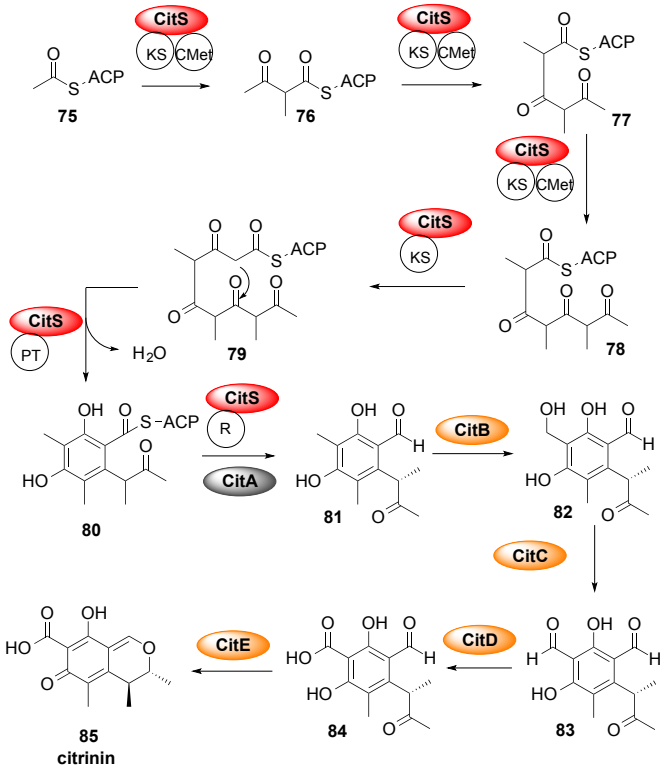

Figure 12 Proposed biosynthesis of citrinin (26,28)

IV. The *Chaetomium*/cochliodone pathway

Few articles were published regarding the *Chaetomium*/cochliodone pathway. Yet, in 2013 Nakazawa *et al.* found the biosynthetic gene cluster of cochliodone A and chaetoglobin A in *Chaetomium globosum* (Figure 13).

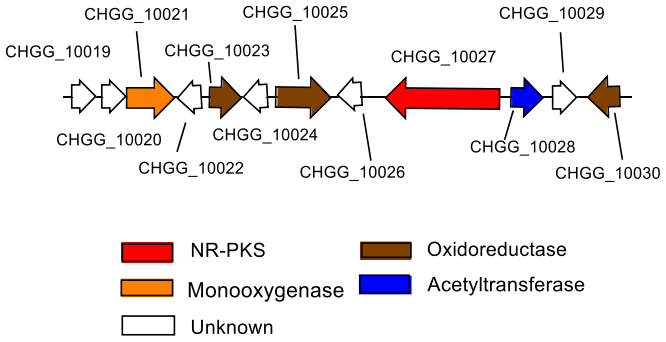

Figure 13 Proposed organization of cochliodones biosynthetic gene cluster in *C. globosum* (30)

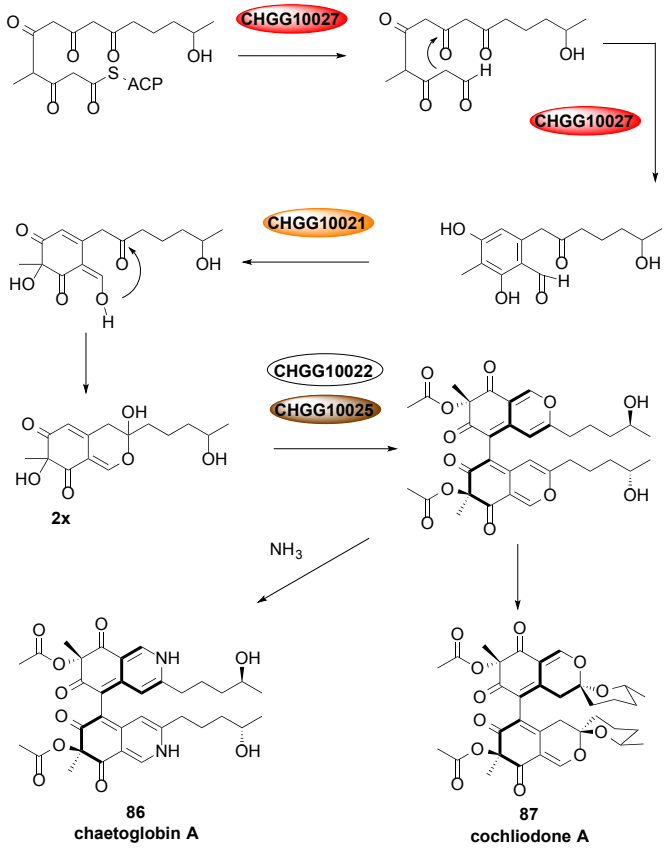

Figure 14 Proposed biosynthesis of cochliodone A and chaetoglobin A (30)

According to their similarity with other known genes and to knock-out experiments, the gene CHGG_10027, a nrPKS, (later named CcdL) is thought to produce the polyketide core by accepting acetyl-CoA as a starting unit and four malonyl-CoA units which could then be transformed by CcdF (CHGG_10021, salicylate 1-monooxygenase), CcdH (CHGG_10023, enoyl reductase), CcdM (CHGG_10028, acetyl transferase) and CcdO (CHGG_10030, enoyl reductase). The acetylation of the alcohol group on the ring system could be achieved by CcdM (CHGG_10028 presumed O-acetyltransferase). Finally, the fungal laccase like multi-copper oxidase encoded by CcdJ (CHGG_10025) is believed to dimerize the cochliodones (32,33).

V. *Hypoxylon* pathway

The *Hypoxylon* pathway was first reported from *Penicillium marneffei*, where two PKS genes *pks12* and *pks11* were described as probably responsible for mitorubrinol and mitorubrinic acid biosynthesis (34). Recently chemical investigation of the stromata of *Hypoxylon fragiforme* led to the isolation of unprecedented bisazaphilones motivating the examination of the *H. fragiforme* genome to suggest their biosynthesis pathways.

By homology with known BGCs encoding azaphilones in *Monascus ruber*, azanigerones in *Aspergillus niger* and mitorubrinol in *Talaromyces marneffei*, two genes clusters among the seven nr-PKS present in *H. fragiforme* were suggested as candidates for the biosynthesis of the isolated azaphilones. This cluster contains also two hydrolases, but no FAD-dependent monooxygenase, P450 monooxygenases, FAD-dependent oxidoreductases and transcription factors. Therefore, Becker *et al.* (35) propose that the two unlinked BGC, Hfaza1 and Hfaza2, act together in order to assemble and diversify azaphilones in *H. fragiforme*.

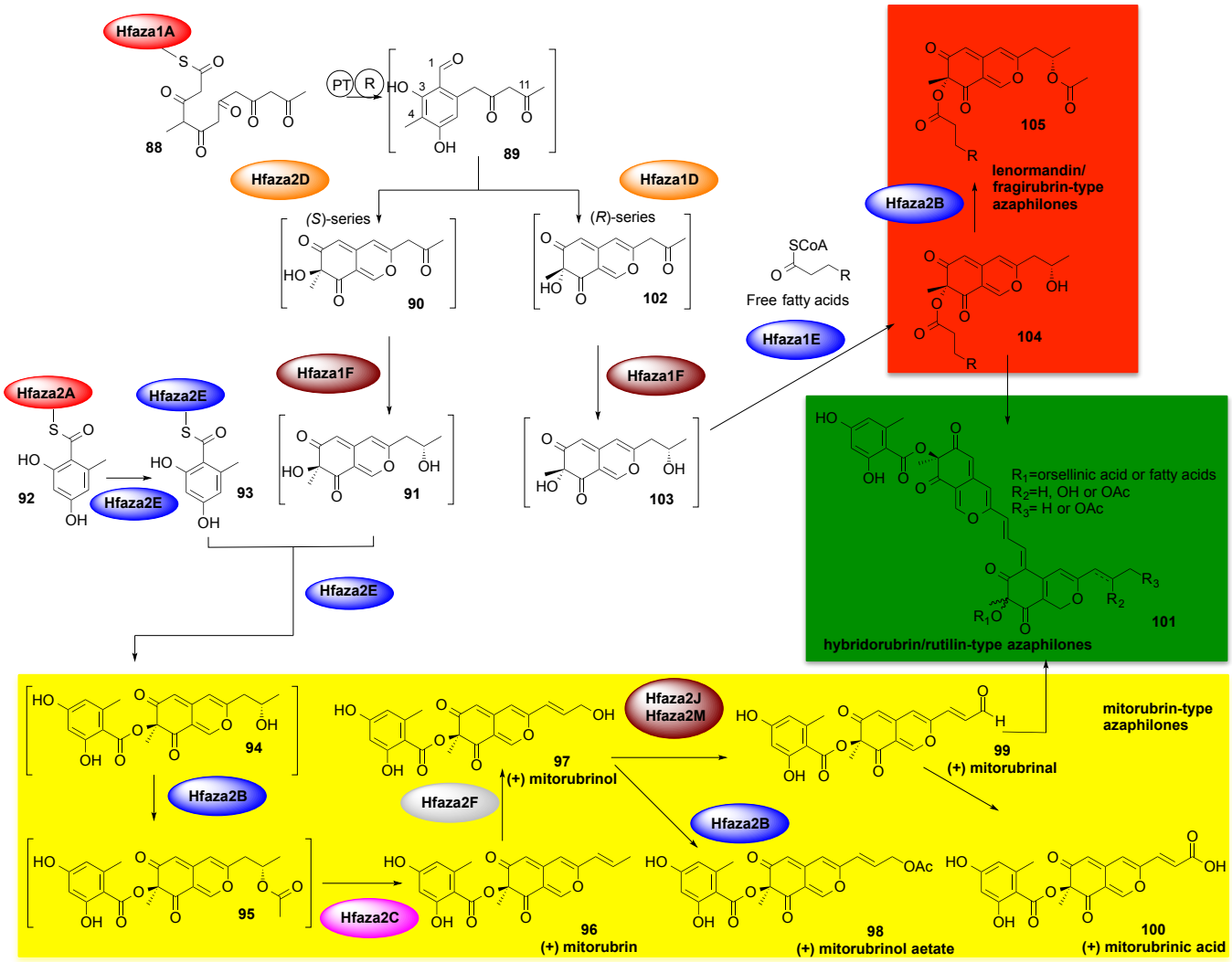

Figure 15 Biosynthetic hypothesis in *H. fragiforme* (31)

Indeed, the first BGC designated as *hfaza1* contains the nr-PKS (Hfaza1A) producing a hexaketide chain **88** cyclized and reduced to produce the reactive benzaldehyde intermediate **89**. Also the FAD-dependent monooxygenases (Hfaza1D or Hfaza2D the latter from the BGC *hfaza2*) could hydroxylate **89** at C-4 follow by the subsequent spontaneous pyran ring formation of **102**. The BGC contains a ketoreductase (Hfaza1F) producing **103**, which may thus undergo acylations by the acyltransferases Hfaza1E and Hfaza2B (from the second BGC) at the C-4 and C-11 alcohols, respectively, to produce lenormandin/fragirubrin-type azaphilones. **104** could also dimerize to form the hybridorubrin/rutilin-type azaphilones (**101**). It is noteworthy that Hfaza1D and Hfaza2D encode a homologous enzyme suggesting they can perform the same reaction. This could therefore be consistent with the observation of different stereoconfigurations at C-4 between mitorubrin-type and fragirubrin-type azaphilones.

A second BGC designated as *hfaza2* contains the nrR-PKS Hfaza2A (SAT-KS-AT-PT-ACP domains) biosynthesizing the orsellinic acid (**92**) added later at C-4 on to the pyranoquinone core by the acyltransferase Hfaza2E, homologous to Hfaza1E and MrPigD, and then acetylated by the putative acetyltransferase Hfaza2B. The deacetylase Hfaza2C might then produce the pyranoquinone ring of mitorubrin (**96**), which would be then successively hydroxylated by

a P450 monooxygenase (Hfaza2F) and oxidized by two FAD-dependent oxidoreductases (Hfaza2J and Hfaza2M) leading to the mitorubrin-type (**96**-**100**) or the hybridorubrin/rutilin-type azaphilones (**101**).

I. Regulation of BGCs

The growing interest towards the azaphilone family led to many attempts to regulate their biosynthetic pathways with the aim to improve their production. The regulation of secondary metabolism in fungi is complex and poorly understood. However, fungal gene clusters may be activated by several ways including epigenetic regulation, transcriptional regulation or stimuli, mimicking environmental stimuli, such as carbon and nitrogen sources, temperature, light, pH, amino acids in the environment, reactive oxygen species, hypoxic conditions, biofilm formation and iron availability, but also stimuli derived from other organisms (36–38). It is noteworthy that BGCs activation is also dependent on the developmental stage of the producing fungus. With regard to azaphilone, some examples have been described.

The regulation of the MonAzPs pathway and/or the citrinin pathway have been the most studied given their importance in food and environmental toxicity, respectively. With the aim of enhancing the metabolic activity of polyketide pathways, several cultivation attempts have been performed such as carbon starvation stress leading to raise the acetyl-CoA pool (39) various light exposition (intensities and colors) (40–42), variation of carbon sources (43–45) or nitrogen (3,44,46–51), different pH (44,52,53), cultivation in high-salt stress conditions (54) or at different temperatures (55). For instance, Guo *et al.* found that the *Monascus* pigments diversity is far more linked to transcriptional regulation than to DNA sequence variation (56). Jun Lv *et al.* discovered that the mycelial morphology was highly correlated to the *Monascus* yellow pigments yield, this was corroborated later on by Chen *et al.* (53,54). Balakrishnan *et al.* found in 2016 that the reductase MrpigH (MppE) when overexpressed, promotes yellow pigment production whereas its inactivation enhanced orange and red pigment. Interestingly enough, Balakrishnan *et al.* highlighted also the fact that the overexpression of MrPigH enhancing the production of yellow pigments was dependent on culture method.(14)

In definitive, comparative transcriptome analysis reveals that several cultivation parameters may contribute to increase the azaphilone yield. Variation of nitrogen sources appear as one of the most important for MonAzPs, although it is important to keep in mind the specificity of the parameters for the considered strain.

With regard to the *Aspergillus* pathway, few studies were conducted on its regulation in particular because of the cryptic nature of the genes. Nevertheless it was found that the overexpression of the transcriptional regulator AzaR activates the *aza* cluster leading to the biosynthesis of several azanigerones not produced in the wild type strain (20). In the same way, the induced expression of the transcription factor gene afoA resulted in the expression of the entire *A. nidulans* asperfuranone cluster which is normally silent and to the production of asperfuranone (57).

In the collaborative way of the azasperpyranone biosynthesis, there might be a transcriptional crosstalk regulation between the two separate BGCs where ATEG_07667 regulated both of the cluster-specific regulators ATEG_03638 and ATEG_07666 allowing activation of their respective BGCs.

The *Chaetomium*/cochliodone pathway has been little studied so far. However, in *C. globosum*, deletion of *laeA* gene, and of the light-regulated developmental factor VeA, leads to modulation of transcription of a number of biosynthetic gene clusters in *C. globosum*. Interestingly, in this strain, the polyketide aureonitol acts like a transcriptional regulator for the biosynthesis of chaetoviridins (33).

Conclusion

This work gives a general understanding on the described azaphilone biosynthetic pathways. Five biosynthetic pathways have been reported in the scientific literature, so far. The MazPs and *Aspergillus* pathways have been thoroughly studied unlike the citrinin and *chaetomium* pathways.

Better knowledge of the azaphilone biosynthesis pathway allowed to identify commons nrPKS and monooxygenases between the different pathways which may be used as specific probes for genome mining approach and that should pave the way towards the identification of promising azaphilones producers. An overall understanding of their biosynthesis pathways will also lay foundation for future genetic manipulation and engineering of the strains and should allow accelerating the discovery and the production of azaphilone lead.

Conflicts of interest

There are no conflicts to declare.

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