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Translating biosynthetic gene clusters into fungal armor and weaponry

Nancy P Keller*

Department of Bacteriology and Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, Wisconsin, USA

Abstract

Filamentous fungi are renowned for the production of a diverse array of secondary metabolites (SMs) where the genetic material required for synthesis of a SM is typically arrayed in a biosynthetic gene cluster (BGC). These natural products are valued for their bioactive properties stemming from their functions in fungal biology, key among those protection from abiotic and biotic stress and establishment of a secure niche. The producing fungus must not only avoid self-harm from endogenous SMs but also deliver specific SMs at the right time to the right tissue requiring biochemical aid. This review highlights functions of BGCs beyond the enzymatic assembly of SMs, considering the timing and location of SM production and other proteins in the clusters that control SM activity. Specifically, self-protection is provided by both BGC-encoded mechanisms and non-BGC subcellular containment of toxic SM precursors; delivery and timing is orchestrated through cellular trafficking patterns and stress- and developmental-responsive transcriptional programs.

Secondary metabolites, or natural products, are produced by a wealth of microorganisms and plants but are particularly abundant in filamentous fungi primarily belonging to the ascomycete taxon Pezizomycotina. Typically the genetic material required to synthesize any particular SM is arranged in a multigene biosynthetic gene cluster (BGC) reminiscent of bacterial operons. Upon synthesis, these metabolites confer a variety of survival functions on the producing organism. These range from protection from abiotic and biotic stresses, such as UV radiation, desiccation, predation from insects and competition with other microbes, as well as participation in metal homeostasis. These small molecules even serve as signals required for differentiation or to direct symbioses or parasitism with other organisms^{1–3}. The very properties that confer survival attributes to the fungus have lent themselves to profitable pharmacological applications and thus ensure continued interest in these fascinating molecules.

Fungal SMs are perhaps best known as potent antimicrobial agents, playing important parts in defense and niche securement for the producing fungus, and these activities have been

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*Correspondence should be addressed to N.P.K. npkeller@wisc.edu.

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directly co-opted for human medicine. For example, penicillin, anidulafungin and griseofulvin are well-known fungal metabolites that have been put into service as antimicrobials. Gliotoxin is produced by *Aspergillus fumigatus* and serves as a virulence factor in invasive aspergillosis⁴. It was first explored in the 1940s as antimicrobial^{5,6}, and its antifungal properties allow its application as an antibiotic marker in fungal transformation⁷. However, gliotoxin and other antimicrobial SMs have also been explored for their potential relevance to other clinical goals. In the case of gliotoxin, its toxic properties have largely precluded use as an antitumor agent, although it is still under consideration for treatment of some cancers^{8,9}. The statins (for example, lovastatin) are efficacious antiparasitic and antifungal agents^{10,11}, yet are routinely used as cholesterol-lowering drugs. Fumagillin and its analogs are used as antifungals in treatment of microsporidial diseases¹², including honey bee colony collapse¹³, but have also shown promise in clinical trials for obesity¹⁴ and cancer¹⁵. Cyclosporin A (also called cyclosporine), although touted for its immunosuppressive properties, is also a potent antifungal; both functions are achieved through targeting the same molecule, cyclophilin, in humans and fungi¹⁶. Finally, the ergot alkaloids are known for their treatment of migraines, beauvericin is employed as an insecticide, and the gibberellins are excellent growth hormones.

To achieve these functional outcomes, fungal SMs act as weapons against a plethora of cellular proteins, many of them present in the SM-producing fungi. As a result, fungi must develop mechanisms to avoid injuring themselves by targeting these same proteins. Alternatively, for SMs that serve as protective armor, fungi must accurately place these molecules in the tissues requiring biochemical shielding. Filamentous fungi are multicellular eukaryotes and, like plants and animals but unlike yeast, differentiate into elaborate and distinct morphologies serving different survival roles. Metabolites providing UV protection of windblown dispersal spores would probably therefore differ from metabolites that have defensive roles, such as protecting against insect predators of sessile fruiting bodies. Elucidation of mechanisms of protection from self-harm and environmental stressors not only unmask basic cellular processes in the producing fungi but may serve to predict possible avenues of resistance in applied therapeutics (for example, development of antimicrobial or cancer cell resistance). This article addresses our emerging understanding of how fungi self-protect from their own metabolites in an orchestrated manner, involving encoded BGC protective devices coupled with cellular trafficking patterns and timed delivery to specific tissue destinations.

Self-protection encoded in the BGC

For fungal SMs that are antifungal agents, the producing fungi must be resistant to their own metabolites or face self-destruction. How do fungi control their own chemicals? The answer can lie in the gene content of the BGC. Genes encoding fungal SMs are clustered into a set of contiguous genes specific for a SM and usually contain all of the activities required for product formation. Although BGCs are often discussed in terms of the biosynthetic modules and tailoring enzymes that determine the chemical structure of the natural product, the clusters also contain gene(s) encoding transcription factors specific for the cluster and 'other' gene(s). These 'other' genes often encode proteins whose activities appear to be incongruous with or unnecessary for formation of the SM and thus are often given limited

attention in descriptions of new biosynthetic gene clusters. However, renewed interest has demonstrated they have important roles in self-protection mechanisms. There are at least three potential in-cluster self-protection strategies—duplication of the SM target (sometimes including SM-resistant forms of the target), detoxification of the SM and efflux of the SM—with many clusters displaying two or more of these properties (Fig. 1).

Duplicated or resistant target

As indicated above, many fungal SMs are valued for specific activities; this specificity of action is linked to the specific biomolecule targeted by the SM. For example, lovastatin blocks cholesterol synthesis by inhibiting HMG-CoA reductase, a crucial step in the cholesterol biosynthetic pathway. This enzyme is also required for synthesis of the fungal sterol ergosterol, explaining lovastatin's antifungal activity¹⁷. To prevent toxicity in the host strain, the *Aspergillus terreus* lovastatin gene cluster encodes a second HMG-CoA reductase proposed to be resistant to lovastatin¹⁸. This motif of an in-cluster HMG-CoA reductase is maintained in related statin SM clusters¹⁹ and can be readily identified in bioinformatics searches.

Fumagillin targets the enzyme methionine aminopeptidase-2, which is required for hydrolytic removal of N-terminal methionine residues from nascent proteins. As with the lovastatin cluster, the fumagillin gene cluster in *A. fumigatus* contains an additional methionine aminopeptidase-2–encoding gene²⁰. Analogous clusters in other genera maintain an in-cluster methionine aminopeptidase-2–encoding gene. It is unknown whether the duplicated enzyme is resistant to fumagillin or simply serves to increase the pool of methionine aminopeptidase-2 such that fumagillin is unable to inhibit all of the enzymes. Finally, cyclosporin A binds to cyclophilin, leading to inhibition of the calcium-calmodulin phosphatase calcineurin. Interestingly, the cyclosporin A BGC encodes cyclophilin²¹, but this gene does not appear to be duplicated in the available *Tolypocladium* genome, suggesting the sequence encoded in the BGC may somehow facilitate resistance to cyclosporin.

These data support a tactic where identification of nonbiosynthetic genes in BGCs might reveal natural product targets and facilitate the search for useful clusters to characterize. Indeed, by searching for a cluster containing the gene encoding mycophenolic acid target IMP dehydrogenase, the mycophenolic acid BGC was identified in *Penicillium brevicompactum*²². A systematic assessment of these target proteins to determine whether and how they are resistant to the BGC SM, as opposed to merely flooding the system with excess target molecules, could aid in our understanding of how the SM disables target activity.

Detoxification

In some clusters, additional enzymes are present that limit the toxicity of SMs by altering their chemical structure (thus changing the ability of the molecule to bind its target) or by eliminating or blocking functional groups important for binding the target. For example, GliT, a gliotoxin oxidoreductase encoded in the *A. fumigatus* gliotoxin BGC (*gli*) modifies the gliotoxin structure to generate a less-toxic molecule^{23,24}.

Although most SM clusters appear to contain all the genes necessary to make, regulate and protect the fungi from the active molecule, there are examples where potential protective activities lie outside of the BGC. GtmA, a gliotoxin *bis*-thiomethyltransferase, lies outside of the cluster and acts to negatively regulate gliotoxin synthesis through production of bisdethiobis(methylthio)gliotoxin, which acts as a *gli* BGC off switch²⁴. Trichothecene mycotoxins are protein synthesis inhibitors produced by many *Fusarium* spp. The acetyltransferase Tri101 acetylates the C3 hydroxyl group of trichothecenes and their precursors making them less toxic to other fungi²⁵, and transgenic plants expressing Tri101 activity show enhanced resistance to the phytotoxic properties of tri-chothecenes^{25,26}. However, deletion of *Tri101* does not inhibit growth of *Fusarium sporotrichioides* on trichothecene-containing medium²⁷.

There are few examples of BCGs encoding detoxifying enzymes at present. This could reflect the tendency to disregard enzymes that do not obviously fit into a biosynthetic scheme. A reinvestigation of such genes and their enzyme activities, for example, *stcT*, encoding a putative glutathione-*S*-transferase in the sterigmatocystin gene cluster²⁸, might reveal some protective or detoxifying function.

Transporters

These membrane proteins are perhaps the best-known way that fungi and other species rid themselves of toxic materials, and thus it is no surprise that BGCs make use of this strategy. Genes encoding transporters (primarily belonging to the ABC superfamily and major facilitator superfamily (MFS)) are commonly found in fungal BGCs. For example, of the 35 BGCs in *A. fumigatus*, 24 contain one or more putative transporters. The transporter gene in the gliotoxin gene cluster, *gliA*, encodes an effective efflux pump important in resistance to gliotoxin²⁴. This mechanism is common for the epipolythiodioxopiperazine (ETP) toxins, including gliotoxin, as other ETPs, such as sirodesmin, also encode self-protective transporters in the cluster²⁹. Tri12 belonging to the MSF-type transporter family affords self-protection to trichothecenes^{27,30}. Transporters can also be coupled to the transcriptional regulation of the cluster as a secondary protection mechanism. Deletion of the gene encoding MSF-type transporter Bik6 in the bikaverin gene cluster results in significant reduction of bikaverin production, mediated in part through a negative transcriptional feedback loop regulating expression of the enzymatic genes³¹.

Some BCG transporters appear to have no impact on SM production or fungal growth³². However, this does not necessarily mean that export and efflux of the SM are unimportant. For example, the gene cluster for the photoactivated perylenequinone toxin cercosporin contains a transporter, yet endogenous resistance to the toxin is provided by two noncluster transporters³³. The roles of BGC-encoded transporters that appear to have no phenotype on the fungus when deleted remain unclear. One possible explanation lies in a redundancy of function.

There is also evidence that transporters can protect fungi from SMs originating from BCGs that do not contain pumps. The zearalenone cluster does not contain a transporter, yet its synthesis in *Gibberella zeae* is dependent on the noncluster ABC transporter ZRA1 (ref. 34). Interestingly, *zra1* expression is positively regulated by the zearalenone in-cluster

transcription factor ZEB2, providing a link between the SM and its protection mechanism even though they are not physically clustered. As ABC transporters are known to display some promiscuity in their substrates, it is likely that many transporters protect from SMs produced by other fungi³⁵. This can be illustrated in *Neurospora crassa*, a fungus sensitive to cer-cosporin that possesses a transporter conferring some resistance to the toxin³⁶, or in the biocontrol fungus *Clonostachys rosea*, which contains an ABC transporter protective against the *Fusarium* mycotoxin zearalenone as well as several fungicides³⁷.

Although the general supposition is that transporters act to export the final BGC product out of the cell, this view misses other potential roles of BGC transporters, including transport of SM precursors into the cell or SM enzymes or pathway intermediates throughout subcellular destinations, as discussed below.

Cellular trafficking

Fungi have not only evolved an impressive set of in-cluster mechanisms to self-protect but also created a complex cellular trafficking highway that compartmentalizes biosynthesis steps along sub-cellular routes, ultimately leading to secretion at the cell surface. BGCs encode many enzymatic steps generating several precursors, some of which exhibit substantial toxicities. Where each enzyme is located and how cargo passes from one biosynthetic step to another presents an engineering challenge for the fungus, and it is becoming increasingly apparent that subcellular compartmentalization is the solution. This strategy appears to provide several aspects crucial for successful SM synthesis, such as access to SM precursor pools, protection from toxic intermediates and trafficking to the secretion machinery.

Cellular detection of secondary metabolite enzymes—chiefly by transmission electron microscopy, vacuole-vesicle fractionation or fluorescence-tagged proteins—has provided a complex pattern of enzyme trafficking through various subcellular compartments^{38,39}. Pathway enzymes can be located in vacuoles, peroxisomes, endosomes, the endoplasmic reticulum (ER) and Golgi apparatus, or the cytosol. Figure 2 illustrates the current understanding of cellular trafficking patterns for three well-characterized SMs: aflatoxin, penicillin and trichothecenes.

Peroxisomes

Peroxisomes, also known as microbodies, are implicated in many cellular processes, including β -oxidation and biogenesis of Woronin bodies (dense organelles plugging septal pores in ascomycete fungi to prevent cytoplasmic bleeding). The first enzymatic steps of aflatoxin and sterigmatocystin synthesis appear to occur in the peroxisome, as do various steps of many other SM pathways, including those responsible for making paxilline, AK-toxin, penicillin, cephalosporin and some siderophores³⁸. Although it has not been demonstrated *per se*, the assumption is that acetyl-CoA generated by β -oxidation may be incorporated into the synthesis of metabolites using acyl-CoA pools as starter or extender units.

Many, but not all, of the SM enzymes localized to the peroxi-some contain peroxisomal localization motifs. Transporters may also be localized to organelles. The non-BGC transporter PaaT, which possesses a peroxisomal binding motif and is involved in penicillin synthesis—presumably through translocation of the side-chain precursors phenylacetic acid and phenoxyacetic acid into the peroxisome—has been characterized in *Penicillium chrysogenum*⁴⁰.

Cytosol

Many SM enzymes localize to the cytosol, a milieu for accruing and trafficking enzymes, substrates and co-factors between subcellular compartments and housing cytoskeleton machinery potentially involved in vesicle movement. Detailed studies of the aflatoxin biosynthetic pathway suggest a transient localization of pathway enzymes in the cytosol during movement of precursors from one subcellular compartment to another (Fig. 2). Additional SMs with at least one step localized to the cytosol include gibberellins, trichothecenes, fumiquinazolines, penicillin and cephalosporin³⁹.

Whether or not these enzymatic reactions specifically require cytosolic conditions or are just in transit to other sites in the cell is largely unknown. Some insight into this question comes from a recent study to improve penicillin yield in *Aspergillus nidulans*. The *A. nidulans* penicillin BGC consists of three genes, encoding the nonribosomal peptide AcvA (located on the cytosolic side of vacuoles), the cyclase IpnA (located in the cytosol) and the final ligase-acyltransferase step by AatA in the peroxisome. Re-engineering the location of IpnA to the peroxisomes results in a complete loss of penicillin synthesis, whereas redirecting the AcvA to the peroxi-some increases penicillin production three-fold⁴¹. Thus, in the case of IpnA, the cytosolic location appears to be critical for function.

Vesicles, vacuoles, endosomes and toxisomes

The intracellular endomembrane network is composed of vesicles and vacuoles that bud from various membranous subcellular compartments. Vesicles are double membrane-bound organelles that can bud from the ER or Golgi apparatus, peroxisome, mitochondria, nucleus and even the cytoplasmic membrane. Endosomes are noted for their ability to receive extracellular materials, recycle molecules and mobilize cargo along the microtubule⁴². Vacuoles, important sites for recycling and storage, are single membrane-bound organelles that can form from fusion of vesicles and endosomes. The discovery of PenV, a vacuolar transporter important in penicillin synthesis, putatively through supplying amino acids to the vacuole-anchored AcvA⁴³, presents a specific example of how materials may be transported from the vacuole to the cytosol (Fig. 2).

Fusion, exchange of material and movement of these organelles allow fungi to direct SM intermediates and enzymes along a cellular pathway to achieve deposition and secretion at cell membranes. Toxisomes, first called aflatoxisomes in aflatoxin synthesis⁴⁴, are products of various vesicle-vacuole fusions that house several biosynthetic steps of SM pathways and provide self-protection from toxic intermediates (Fig. 2). Final export from toxisomes (or any cellular compartment) out of the cell is not clear but hypothesized to involve exocytosis and/or active transport.

Regardless of how BGC materials are trafficked through the fungus, the impact of high production of a SM on hyphal morphology can be substantial. A case in point is illustrated by a study showing massive alterations in *Aspergillus parasiticus* hyphal morphology during aflatoxin-inducing conditions where a high proportion of subcellular materials are tied up in aflatoxin-some biogenesis⁴⁵.

Expression pathways and delivery to specific tissues

SMs are, by definition, molecules that are not needed for routine cellular function; thus, fungi do not and cannot sustain continuous flux to SM pathways without cost to growth and development. Synthesis should be optimized and occur only during conditions requiring their use. Furthermore, SMs may actively and preferentially accumulate in specific tissues. Filamentous fungi differentiate into several morphologies in response to the environmental milieu and, as a result, SM localization can be specific to asexual or sexual spores, hyphae or resistant survival structures (for example, sclerotia, which are large masses of hardened, tightly adhered mycelium able to survive environmental extremes and insect predation). How are these aspects of secondary metabolism achieved? Here transcriptional cascades, which can include the transcription factors encoded in many BGCs, become of central importance.

Expression of BGCs in response to stress

Numerous reports have demonstrated that fungi synthesize SMs in response to environmental stress. For example, BGC activation occurs during fungal encounters with other microbes and insects, and a particular coupling exists between oxidative stress pathways and transcriptional activation of BGCs^{46,47}.

Basic leucine zipper (bZIP) proteins are the primary conduits linking secondary metabolism with oxidative stress. bZIP proteins, well characterized in *Saccharomyces cerevisiae* as yeast activator protein (Yap) transcription factors⁴⁸, are crucial for fungal responses to several types of abiotic stress, most notably oxidative (ROS) and osmotic stress. These proteins, including the two canonical ROS response bZIP proteins AtfA/Atf1 and NapA/Yap1 are also critical mediators of the SM response in filamentous fungi (Table 1). Some BGCs are regulated positively and some negatively by bZIP proteins, and this regulation is dependent on environmental conditions. The reasoning behind the linkage of stress responses with altered secondary metabolism is several-fold and involves the hypotheses that some SMs (for example, aflatoxin) protect from ROS damage, that coupling several modes of protection (for example, antioxidant enzyme and toxin synthesis) under one bZIP regulon is efficient, and that, in the case of SMs negatively regulated by bZIPs, suppressing their synthesis allows for valuable energy to be shunted to other critical responses specific for the particular stress at hand.

As mentioned above, studies have also shown that encounters with other microbes or insects can induce BGC expression and metabolite production in fungi^{49,50}. These SMs can function as antimicrobials or as feeding deterrents and toxins toward insects. There is evidence that some of the bZIPs regulating secondary metabolism in fungi are also important in the response toward other organisms. *Aspergillus napA*—involved in negatively

regulating a subset of *A. nidulans* SMs (Table 1)—is induced by *Pseudomonas aeruginosa* redox SMs and required for a defensive sporulation response in this genus⁵¹. *Aspergillus* RsmA, a positive regulator of the feeding deterrent sterigmatocystin, confers protection from the fungivore *Folsomia candida*, presumably through providing a chemical shield⁵². RsmA directly regulates the sterig-matocystin BGC transcription factor AfIR by binding to a Yap site in the *aflR* promoter; mutations of this site result in strains with greatly reduced *aflR* expression and sterigmatocystin synthesis.

Our knowledge of what ligands trigger BGC responses to abiotic and biotic stressors and the transcriptional pathways transmitting the triggers is far from complete. LaeA and VeA, members of the conserved fungal velvet complex linking morphological development with light, play a role in many of these cascades as do nitrogen⁵³ and metal⁵⁴ imbalances and epigenetic modifications of the chromatin code⁵⁵. Contrasting and comparison of the transcriptional profiles of fungi under different environmental milieus and organism encounters may allow the identification of other cascades with specificity toward BGC expression.

Developmental delivery of SMs to spores

Most fungi disperse by airborne asexual spores that can travel long distances at considerable altitudes, where exposure to UV radiation is significant. Aromatic and pigmented SMs—often called melanins—have been shown by several studies to protect spores from UV damage^{56–58}. In radiotrophic fungi, some evidence suggests that melanins function to enhance fungal growth via capture of ionizing radiation for energy conversion⁵⁹. Spore pigments can also be critical in the development of normal asexual and sexual spore structures^{60,61}. Deletion of sporulation-specific BGC genes can result in absolute loss of sexual development in fungi, as seen in the requirement for a polyketide for fertile structures in *Sordaria macrospora*⁶² or the contribution of numerous SMs to development of sclerotia (sites of sexual sporulation and resistance structures to insect feeding) in *Aspergillus flavus*^{63–65}.

Although the linkage with SM synthesis and sporulation has been long noted⁶⁶, it is only recently that a process for this journey has been elucidated. Asexual spore development in *Aspergillus* and *Penicillium* species is mediated by the transcription factor BrlA⁶⁷. Deletion of *brlA* yields a fungus unable to produce asexual spores, although hyphal and sexual spore production remains intact. Coupled with the loss of asexual spores is the loss or diminishment of a subset of SMs in BrlA-null mutants⁶⁸. A compilation of studies using *brlA* mutant strains and/or spore extraction techniques have led to the identification of several *A. fumigatus* SMs specific to the asexual spore, including dihydroxynaphtalene (DHN) melanin, endocrocin, trypacidin, fumigaclavine and fumiquinazoline C^{69–73}. Interestingly, with the exception of trypacidin, these spore clusters lack a BGC transcription factor, which may facilitate efficient regulation by BrlA.

An understanding of the transcriptional machinery required for production of SMs specifically in the spore first emerged from the finding that all five of the characterized *A. fumigatus* spore BGCs and *brlA* were transcriptionally regulated by LaeA, a global regulator of fungal secondary metabolism⁷⁴. This allowed for a model where LaeA regulation of spore

metabolites was indirect and mediated through BrlA (Fig. 3). This hypothesis was examined by dissecting fumiquinazoline C synthesis in *A. fumigatus*. A complete vision of fumiquinazoline C placement to the asexual spore cell wall was achieved by coupling fluorescence microscopy with metabolic profiling of *fmq* (fumiquinazoline) cluster and *brlA* mutants over a timeline of fungal development⁷³. The journey requires BrlA regulation of gene expression and conidiophore formation, trafficking of *fmq* enzyme or precursor machineries from a toxisome-like origin through the cytosol to the ER or Golgi apparatus and ends with localization of FmqD, the terminal enzyme of the fumiquinazoline biosynthetic pathway, to the spore wall (Fig. 3).

The joint regulation of other spore SMs by LaeA and BrlA defines the transcriptional regulatory cascades leading to production of these metabolites in the spore but does not reveal the cellular trafficking patterns leading to secretion from the cell wall (or in the case of DHN melanin, incorporation into the cell wall). These pathways are likely to be as varied as the trafficking patterns illustrated for aflatoxin, trichothecenes and penicillin (Fig. 2) and involve shuttling to various subcellular compartments. Certainly cell biological studies will help clarify the patterns and mechanisms of placement of specific SMs to specific tissues— asexual spores as well as sexual tissues and resistant morphologies.

Conclusion

Canonical fungal BGCs often contain not only the genetic material to synthesize SMs but also mechanisms to self-protect from injurious bioactive properties of these metabolites. These mechanisms reveal clues of SM activity targets and can be predictive of modes of resistance in future applications of these compounds. Self-protection may also underlie the emerging cell biological studies illustrating variation in subcellular compartmentalization and trafficking of SM pathway precursors and enzymes. These studies are in their infancy, with promise to expand into the role of molecular motors in intracellular transport of SM machinery through the fungal thallus. However, cellular trafficking alone cannot ensure that SMs are produced at the right time and in the correct tissues; transcriptional programs that dictate when and where BGCs should be expressed are also coupled to these activities.

Advances in genetics, chemistry and cell biology have allowed for a clearer understanding of the process of specialized metabolism in filamentous fungi and also some insight into why the yeast genome, a fungus exhibiting little deviation in morphologies, is devoid of BGCs. Future studies are likely to continue to fill in holes in the burgeoning field of fungal secondary metabolism. By taking advantage of the modes of BGC self-protection described here, bioinformatic examination of the vast number of sequenced ascomycete genomes will most assuredly provide new information on SM targets and potential mechanisms toward developing resistance to SMs of pharmacological interest. Subcellular compartmentalization, which has a critical role in successful synthesis of SMs, needs further study and may present roadblocks in heterologous expression of fungal BGCs in bacteria or yeast, where cellular compartments may be lacking or inadequate. Finally, the integration of transcriptional cascades governing fungal differentiation processes and responses to environmental signals promise to tie into BGC activation in a logical and reasoned manner that should help address the challenge of activation of cryptic gene clusters.

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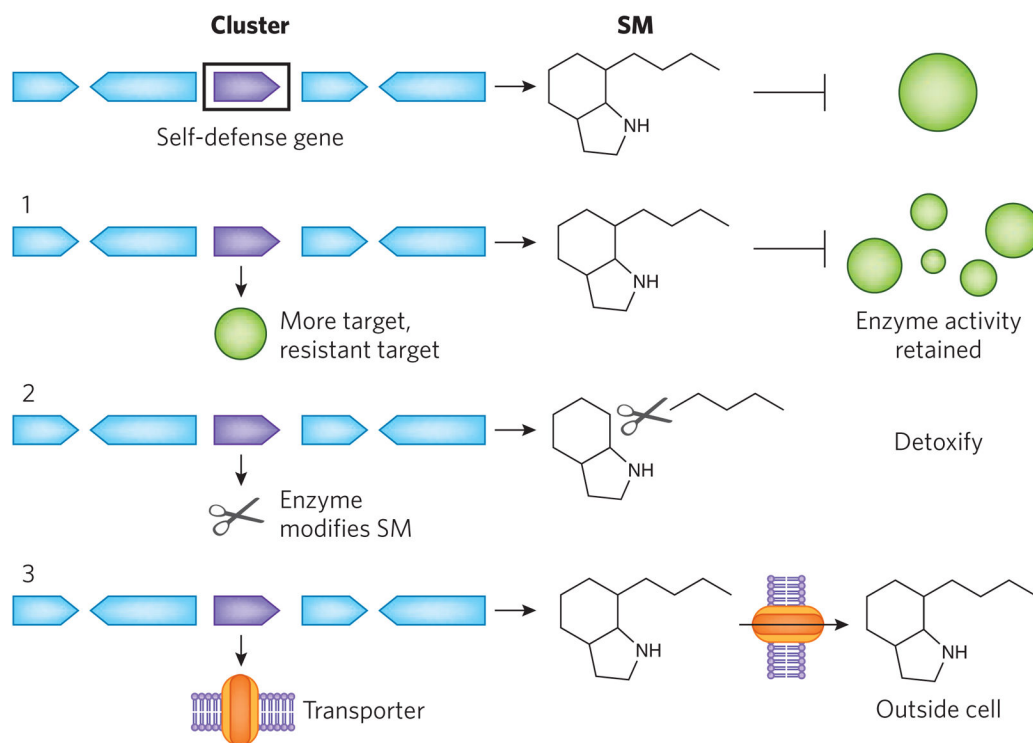


Figure 1. Fungal BGCs can contain genes encoding one or more self-protective devices
 Duplication (1) of the target of the BGC product can occur as a resistant form of the target that sustains activity even when the target is inhibited or in a form that is still sensitive but increases the target pool, allowing for some escape and, hence, activity. Other devices include enzymes that chemically modify the BGC product to fully or partially detoxify it (2) and transporters that export the BGC product outside of the cell (3).

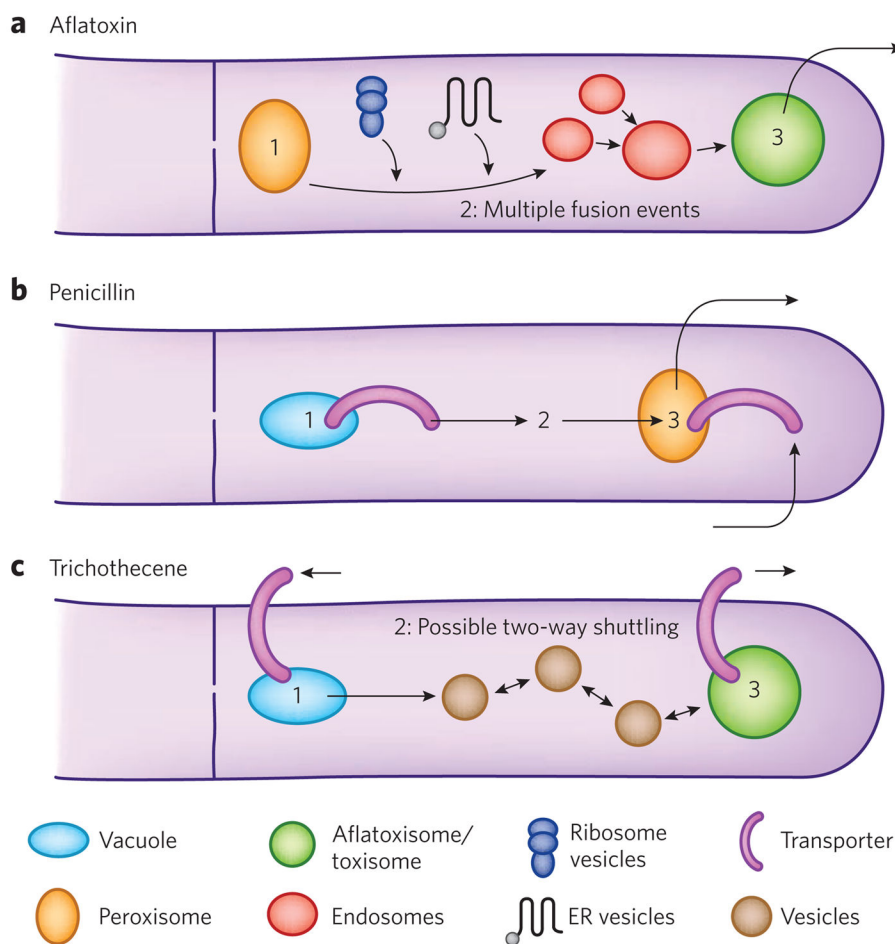


Figure 2. Subcellular trafficking models for biosynthesis of aflatoxin, penicillin and trichothecene

(a) Aflatoxin synthesis is proposed to originate in the peroxisome (1); synthesis then proceeds from this organelle through multiple fusion events incorporating materials from the ER and ribosomally derived vesicles (2) to yield endosomes that fuse, eventually leading to large bodies containing substantial concentrations of aflatoxin (3). Contents of the aflatoxisomes are released to the environment in a yet unidentified manner. (b) Penicillin synthesis originates at the interface of the vacuole and cytosol (1). The first intermediate is released in the cytosol and processed by a second cytosolic enzyme (2), and the product from the second enzyme is transported to the peroxisome, where synthesis is completed (3). Two transporters, one on the vacuole and one on the peroxisome, are required for synthesis. The final product is released to the environment in a yet unidentified manner. (c) Trichothecene synthesis probably originates in a vacuole (1), with multiple steps associated with vesicle fusion events (2), with sufficient fusions leading to a toxisome (3). An in-cluster transporter is proposed to release the end product from the toxisome and outside of the cell but may also act to transport materials into the cell and between vesicles^{38,39}.

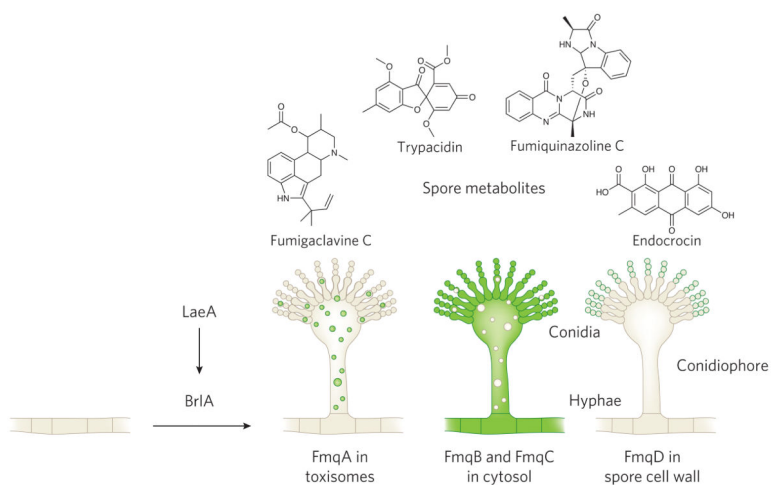


Figure 3. A transcriptional conduit from LaeA to BrIA regulates production of spore secondary metabolites

BrIA induction results in morphological development of the asexual sporulation structure (the conidiophore) and expression of spore-specific BGCs, including the *fmq* BGC genes responsible for making fumiquinazoline C. *fmq* genes are expressed only when BrIA is active, with FmqA localizing to a toxisome-like structure, FmqB and FmqC in the cytosol, and FmqD, the terminal enzyme, localizing to the spore cell wall⁷³.

Table 1

SMs regulated by bZIP proteins

Fungus	SM	bZIP	Ref.
<i>Aspergillus parasiticus</i>	Aflatoxin	AtfB	75
	Aflatoxin	Apyap1	76
<i>Aspergillus nidulans</i>	Sterigmatocystin	RsmA	52
	Asperthecin	RsmA	52
	Sterigmatocystin	NapA	77
	Emericellin	NapA	77
	Asperthecin	NapA	77
	Shamixanthone	NapA	77
	Epishamixanthone	NapA	77
	Various	AtfA	78
<i>Pestalotiopsis fici</i>	Isosulochrin	PfzipA	79
	Ficipyronea	PfzipA	79
	Iso-A82775C	PfzipA	79
	Pestaloficiol M	PfzipA	79
	Res1214-1	PfzipA	79
<i>Aspergillus fumigatus</i>	Pseurotin	HapX	54
	Fumagillin	HapX	54
	Fumitremorgin	HapX	54
	Hexadehydroastechrome	HapX	54
	Gliotoxin	RsmA	80
	Gliotoxin	FlbB	81
<i>Fusarium graminearum</i>	Trichothecenes	Fgap1	82
	Deoxynivalenol	Atf1	83
<i>Fusarium fujikuroi</i>	Bikaverine	MeaB	84
<i>Aspergillus flavus</i>	Aflatoxin	MeaB	85
<i>Botrytis cinerea</i>	Botrydial	Bcatf1	86
	Botcinic acid	Bcatf1	86
	Abscisic acid	Bcatf1	86
<i>Aspergillus ochraceus</i>	Ochratoxin	AoYap1	87