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A Global View of Metabolites

Fungi are rich sources of medically useful, as well as toxic, secondary metabolites. In this issue of *Chemistry & Biology*, Bok et al. [1] show that, in *Aspergillus nidulans*, microarray analysis and manipulation of a global regulator can identify novel metabolite genes.

Fungi are well known for their vast diversity of secondary metabolites [2], including important antibiotics, such as penicillin produced by *Penicillium* and *Aspergillus* species, and dangerous toxins, such as aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*. Some secondary metabolites produced by human pathogens, such as melanin in *Aspergillus fumigatus*, are important for parasitism [3]. Likewise, many plant pathogenic fungi, such as the southern corn leaf blight agent *Cochliobolus heterostrophus*, produce toxins that are responsible for the major disease symptoms [4]. Indole alkaloids produced by the ergot fungus (*Claviceps purpurea*) are among the most potent neurotropic agents known and can cause psychosis and death in humans and livestock, whereas the aflatoxins, the most carcinogenic of metabolites, pose considerable long-term danger to humans [2]. These natural products represent broad structural and biosynthetic diversity, including terpenoids, polyketides, peptides, and alkaloids. Synthesis of these metabolites is typically regulated, probably to ensure that the energy and nutrients used in their synthesis are expended only in the environments where the metabolites are beneficial.

Identification of genes involved in producing secondary metabolites is important for several reasons. Enzymes for their biosynthesis can give us information on the mechanisms of metabolic transformations, which can be used in turn to direct synthesis of new and useful metabolites [5]. Also, once key toxins in human, plant, or animal disease are identified, their modes and sites of action can be elucidated, enabling more informed or directed therapy. Furthermore, discovery of new antibiotics, antiviral compounds, anticancer compounds, and so forth, and their development into pharmaceuticals, can be facilitated by such knowledge [6]. Importantly, once the gene for one biosynthetic step has been identified in the fungal genome, adjacent genes are likely to encode other enzymes of the same pathway [7]. Thus, the tendency of fungi to cluster genes for a common pathway helps considerably in gene discovery, although identifying that first gene can be very laborious. Available genome sequences should afford more facile means to identify novel secondary metabolism gene clusters and, ultimately, novel metabolites.

Some transcriptional regulator genes have been associated with biosynthesis genes and act specifically on individual pathways, whereas other more global regulators may coordinate secondary metabolism with growth or developmental stages [2, 8]. In this issue, Bok et al. [1] manipulate the *Aspergillus nidulans* gene *laeA,* a global regulator of sterigmatocystin (related to aflatoxins), lovastatin, and penicillin biosynthesis, and observe effects on previously uncharacterized clusters of genes. Microarray-based transcriptional profiling of a *laeA*-deletion and a *laeA*-overexpression strain revealed discrete segments of the genome that are subject to *laeA* regulation. One such cluster was verified as being involved in secondary metabolism by mutation of one of its genes, with the resulting loss of production of the indole-alkaloid terrequinone A.

Regulation of secondary metabolism is tightly linked to developmental regulation in *Aspergillus* species [2]. Therefore, alterations in master regulators have pleiotropic effects. The *A. nidulans fadA* gene product, a G_{α} protein, regulates conidiation through its effect on *brlA*, and regulates penicillin and sterigmatocystin synthesis through effects on pathway-specific regulators such as AflR (which controls sterigmatocystin genes), as well as global regulators such as protein kinase A (PkaA). The latter suppresses LaeA activity, though it is unknown whether this is a direct or indirect effect (there is no indication of a PkaA target site in LaeA) [8]. Interestingly, *laeA* is regulated by the pathway-specific regulator AflR, providing feedback that may prevent overexpression of sterigmatocystin [8].

One model suggests that LaeA may participate in, or affect, histone methylation, and this might alter the state of chromatin in metabolite gene clusters [2]. A conversion from a heterochromatic to euchromatic state would be expected to upregulate the clustered genes. Currently, this is a speculative hypothesis. The LaeA sequence suggests it may bind *S*-adenosylmethionine, although conserved domain searches do not give especially low expect values (personal observation; $\geq 6 \times 10^{-6}$ to the signature domain of an *O*-methyltransferase). Also, no obvious DNA binding motif is evident in the LaeA signature, suggesting that it might need to be recruited to the chromatin by DNA binding proteins. If so, the basis for LaeA specificity for secondary metabolism gene clusters is not obvious. On the other hand, an N-terminal fusion of LaeA to green fluorescence protein localizes to nuclei [8], suggesting that its effect on mRNA levels may be a direct modulation of transcription.

The approach taken by Bok et al. [1] is an elegant utilization of genomic resources in an extraordinarily well-characterized regulatory system. An interesting question now is whether the approach can be extrapolated beyond *Aspergillus* species to the myriad other fungi that are metabolically interesting but for which there is much less information on gene regulation. In such fungi, the identification of characteristic biosynthetic genes may be an easier approach. Most known secondary metabolite gene clusters are characterized by genes for one or more of four protein families: nonribosomal peptide synthetases, polyketide synthases, terpene cyclases, and DmaW-homologous prenyl transferases [2]. It is noteworthy that the prototypical *dmaW*, whose product catalyzes the first step in ergot alkaloid synthesis, was eventually identified after decades of investigation by several groups, culminating in purification and

partial protein sequencing and degenerate-primer PCR [9], and that once identified its sequence showed no relationship to any other known prenyl transferase. Yet *dmaW* homologs are evident in numerous secondary metabolite clusters, including the newly identified terrequinone A cluster [1]. In the future, other previously unrecognized, but essential and conserved, gene families might be identified for other metabolite gene clusters. For example, the loline alkaloid (*LOL*) gene cluster of *Neotyphodium uncinatum* contains ten genes, none of which fall into the four aforementioned categories [10]. Even so, the relationships of *LOL* proteins to pyridoxal phosphate enzymes, monooxygenases, or nonheme-iron oxygenases strongly suggest that this is a secondary metabolism cluster.

Thus, two approaches appear promising for identifying novel secondary metabolism clusters in sequenced genomes: identification of signature protein families [2], and demonstration of coordinated regulation with other metabolite gene clusters [1]. Bok et al. [1] demonstrate that the latter approach holds considerable promise for *Aspergillus* species. The degree to which the technique can be applied to other fungal groups depends on whether global regulation is typical or atypical across the kingdom. A promising observation is that homologs of *laeA* are apparent in sequenced genomes of other fungi in the phylum Ascomycota. But, there is actually an embarrassment of riches. For example, a BLASTp search of LaeA sequence against the protein database for *Gibberella zeae* PH-1—also known for its secondary metabolites and toxins—brings up 37 hits with E values ranging from 1×10^{-47} to 7×10^{-21} (personal observation). Obviously, these cannot all be global regulators. Does the top of this list represent the functional homolog of LaeA, or does the next one (at 9.7×10^{-46}), or do none of them? Follow-up studies of other systems will have evolutionary implications as well. If global regulation appears to be typical of diverse fungal genera, what ecological conditions might they have in common that would have selected this behavior? If, on the other hand, other fungi tend not to exhibit global regulation of secondary metabolism, what are the ecological variables that have led to such divergent regulatory strategies?

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Selected Reading

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A Tighter RVxF Motif Makes a Finer Sift

Most partners of protein phosphatase 1 rely on an instance of the so-called RVxF motif for interaction with the enzyme. In this issue of *Chemistry & Biology*, a stringent definition of the motif targeting high-affinity instances enabled Meiselbach and colleagues to recognize novel binding partners with high specificity [1].

For a protein, a key to durable protection from mutation is the acquisition of one or more essential functions that involve interactions with multiple partners. Few proteins are better placed to illustrate the success of this strategy than protein phosphatase 1 (PP1), an enzyme that removes phosphoryl moieties from Ser or Thr residues in proteins [2–5]. Beginning in early eukaryotic evolution, this enzyme formed interactions with proteins that target and regulate its activity toward various specific substrates. Some of the earliest of these alliances, presumably including those with the mitotic regulator Sds22 [6] and the less well characterized Inhibitor-3 [7], became indispensable for eukaryotic survival. The ensuing need for conservation of the different interaction sites involved has severely constrained further mutation of the enzyme [2].

However, the structural rigidity of PP1 has not halted its functional evolution. To the contrary, to meet new regulatory demands in different eukaryotic lineages, its