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Evolution of modular conidiophore development in the aspergilli

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Conidiophores are reproductive structures that enable filamentous fungi to produce and disseminate large numbers of asexual spores. The diversity in conidiophore morphology is sufficiently large to serve as a basis for fungal systematics. *Aspergillus* and *Penicillium* species are members of the family Trichocomaceae that form conidiophores with characteristic architecture. Whereas the *Penicillium* conidiophore appears to be a modified branched hyphal structure, the *Aspergillus* conidiophore is seemingly more complex and includes additional cell types. Here, it is proposed that the "aspergillioid" conidiophore may have evolved from a "penicillioid" ancestor via changes in expression of key regulators of the cell cycle and the GTPase Cdc42. Because the transcriptional regulatory network that controls conidiophore development in *Aspergillus* is well characterized, further study of how this network links to regulators of the cell cycle and Cdc42 should provide fundamental insight into the evolution of developmental morphogenesis in fungi (i.e., fungal evo-devo).

Keywords: conidiophore; *Aspergillus*; *Penicillium*; cell cycle control; Cdc42 GTPase module

Understanding the evolution of developmental and morphological processes constitutes one of the primary goals of the field of "evo-devo" (evolution of development). This field has generated significant insight into the molecular mechanisms that cause morphological variation in both animals and plants. One key concept to emerge is the role that changes in gene regulatory sequences play in producing varied morphologies.[1] The resulting temporal and/or spatial alterations in the expression of key genes that determine cell shape and division patterns often trigger profound differences in developmental outcomes. By comparison to animals and plants, remarkably little is known about the evolution of development in the fungi. This is particularly surprising given the wealth of resources and tools that are available for studies of fungal evo-devo. For example, more complete genome sequences are available for fungi than for either animals or plants, and it is far simpler to manipulate individual fungal genes.[2–4] Moreover, fungi produce a striking array of fruiting structures (e.g., mushrooms) that presumably reflect the operation of diverse developmental programs. One central question is whether change in the expression of key morphogenetic genes underlies fungal development to the same extent as it does in animals and plants.

The Trichocomaceae are a family of fungi found within the order Eurotiales.[5] This family contains the well-studied genera *Aspergillus* and *Penicillium* (note that a recent study has proposed that the Trichocomaceae be split into three families: Aspergillaceae, Trichocomaceae, and Thermoascaceae[6]). These genera develop asexual reproductive structures (i.e., conidiophores) that are sufficiently unique to be taxonomically informative. Notably, conidiophore architecture becomes more complex when the anamorphic genus *Paecilomyces* is compared to *Penicillium* and *Aspergillus* (Fig. 1). In *Paecilomyces*, conidiophores are essentially aerial septate hyphae that are branched, with each branch terminating in a phialide that generates spores in a basopetal pattern. *Penicillium* conidiophores consist of septate aerial hyphae that are branched, but are more complex in that each branch terminates in a whorl of phialides. *Aspergillus* conidiophores

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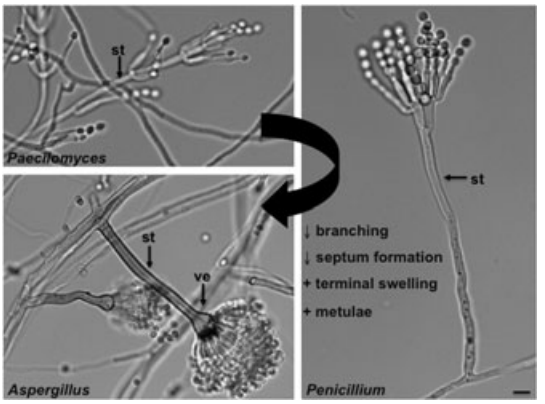

Figure 1. Conidiophore morphology in the *Trichocomaceae*. Examples of conidiophores produced by *Paecilomyces*, *Penicillium*, and *Aspergillus*. The *Paecilomyces* and *Penicillium* isolates have not been identified to the species level. The *Aspergillus* isolate is *A. nidulans* strain FGSC A4. The black arrow denotes the increased complexity of conidiophore morphology. The morphological alterations associated with evolution of the "aspergillioid" conidiophore morphology are indicated (st = stalk, ve = terminal vesicle). Conidiophores were imaged using the "sandwich slide" technique.[25] Bar, 3 μm.

feature several additional modifications that contribute to their increased complexity, including (i) the suppression of branching and septum formation in aerial hyphae (i.e., the stalk), (ii) the formation of a terminal swelling on the stalk that is known as the vesicle, and (iii) in some species (i.e., those that are biseriate), the introduction of an additional tier of cells known as metulae, which in turn bud to generate phialides.

Among the Trichocomaceae, the model fungus *A. nidulans* is widely recognized for its ease of genetic and postgenomic manipulation.[7,8] As a result, considerable progress has been made toward understanding the regulatory network that controls conidiophore development in this fungus.[9–11] Chemical signals (e.g., FluG, Psi factors) and light are two key environmental stimuli that govern the transition from hyphal growth into conidiophore development.[12,13] A network of regulatory factors known as upstream developmental activators (UDAs) is expressed during this transition (Fig. 2). Besides acting to terminate hyphal growth and activate development, these factors (i.e., FlbB, FlbE, FlbD, and FlbC) also collectively promote the expression of the central developmental pathway (CDP; Fig. 2). The pivotal component of the CDP is the transcription factor BrlA, which activates the transcription factors AbaA and WetA in a pathway that includes multiple feedback loops and is regulated by additional modifiers (i.e., MedA and StuA). Notably, BrlA drives the formation of all cell types up to and including phialides, whereupon AbaA is needed for proper phialide function.[14] Most UDAs and components of the CDP are conserved in other *Aspergillus* spp., as well as in *Penicillium* spp., which implies that the overall architecture of this regulatory network has been conserved within the Trichocomaceae.[10,15] However, the downstream effectors of the UDAs and the CDP are not well characterized. In *A. nidulans*, the expression of genes involved in secondary metabolism and spore maturation appears to be directly regulated by BrlA and/or AbaA.[16,17] Nevertheless, although conidiophore development necessitates dramatic changes in the morphogenetic and cell cycle programs that normally operate in growing hyphae, it is not clear how the UDAs→CDP regulatory network triggers these changes.

A. nidulans has been used as a model fungus for understanding fundamental features of the cell cycle and hyphal morphogenesis.[18,19] These studies have exploited a wealth of available mutants defective in diverse aspects of cell-cycle progression or morphogenesis. The characterization of developmental defects in these mutants has yielded some insight into the identities of the factors that could potentially underlie the evolution of conidiophore morphology in the Trichocomaceae. For example, a mutation affecting the *A. nidulans* homolog of the tyrosine kinase Wee1 (i.e., AnkA) causes dramatic defects in conidiophore architecture (Fig. 3). These include the appearance of septa in the stalk, the pronounced absence of a terminal vesicle, and the failure to properly undergo the transition from "hyphal-like" growth of the stalk and vesicle to "yeast-like" growth of metulae and phialdes. Wee1 is known to phosphorylate the tyrosine-15 (Y15) residues of the

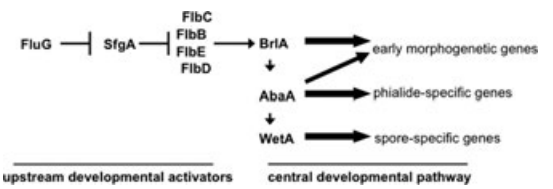

Figure 2. Regulatory network that controls conidiophore development in *A. nidulans*. Note that FlbB and FlbE function as partners to regulate BrlA. See the text for further details.

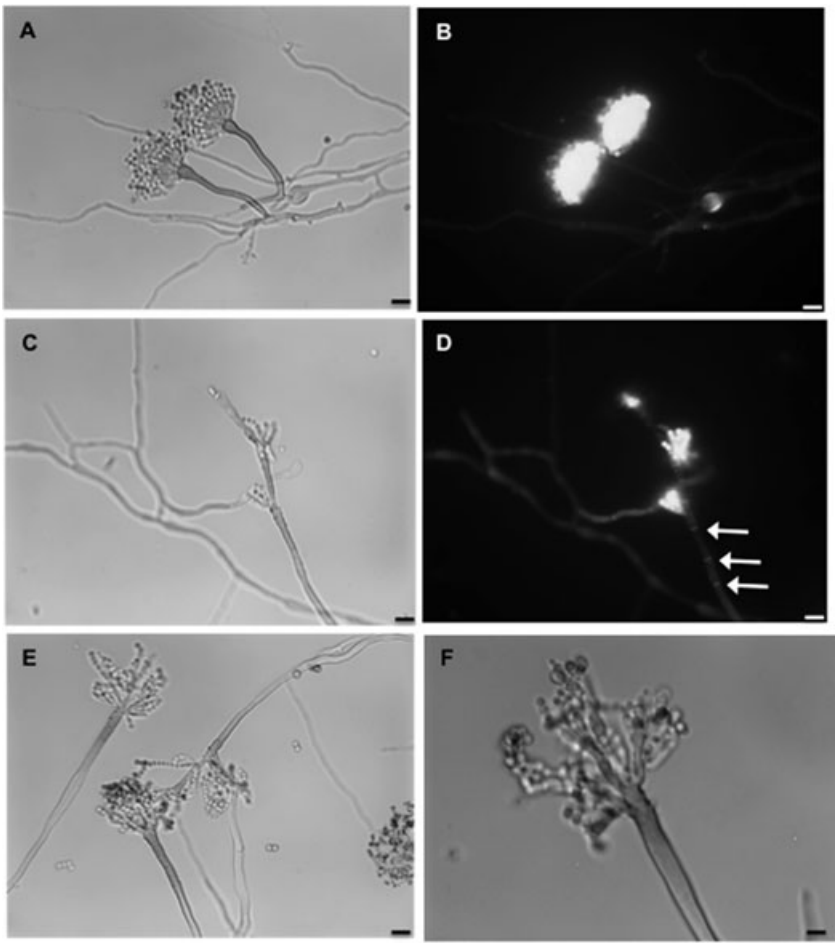

Figure 3. Loss of AnkA function alters conidiophore morphology in *A. nidulans*. (A, B) Wild-type (strain FGSC A28) conidiophores. (C–F) Representative examples of *ankA* mutant (strain APK35) conidiophores. The *ankA* allele in APK35 was originally named *sntA1*.[26] Samples in panels B and D are stained with calcofluor white. Arrows in panel D denote septa present in the conidiophore stalk. Bars, 5 μm, except 2 μm in panel F.

cyclin-dependent kinase CDK1 (i.e., NimX in *A. nidulans*), and in doing so maintains CDK1 in an inactive state during the G2 phase of the cell cycle (Fig. 4).[20] The removal of this phosphorylation by the phosphatase Cdc25 (i.e., NimT in *A. nidulans*) is a requirement for mitotic entry.[20] Accordingly, the conidiophore defects caused by a mutation in AnkA (or by mutation of the Y15 residue of NimX[21]) imply that imposition of a G2 cell cycle delay is needed to inhibit septum formation in the stalk and permit formation of the terminal vesicle. Additional studies show that the ability to precisely regulate the small GTPase Cdc42 is required for normal conidiophore development. RgaA is the *A. nidulans* homolog of the yeast GTPase-activating protein (GAP) Rga1, which coverts Cdc42 from its active GTP-bound state to an inactive GDP-bound state (Fig. 4).[22] Thus, the absence of Rga1 results in Cdc42 hyperactivity and defective morphogenesis. In *A. nidulans*, *rgaA* deletion mutants generate abnormal conidiophores that do not possess a terminal vesicle and form metulae that resemble hyphae (S. Harris, unpublished results). Moreover, the formation of lateral branches is no longer suppressed in these mutants. Genetic analyses demonstrate that these defects likely result from improperly regulated Cdc42 activity. These observations suggest that Cdc42 activity must be downregulated at two distinct points during conidiophore development: (i) at the tip of stalk to stop polarized growth and

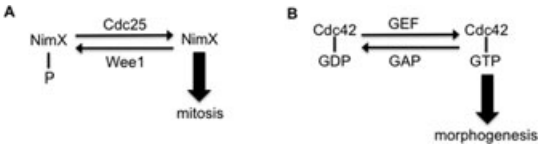

Figure 4. Regulatory modules implicated in the control of morphogenesis during conidiophore development. (A) The CDK module. Wee1 maintains CDK in an inactive phosphorylated state. (B) The Cdc42 GTPase module. Rga1 inactivates Cdc42 by promoting its intrinsic GTPase activity. See the text for further details.

enable formation of the terminal vesicle, and (ii) following the emergence of metulae from the terminal vesicle to facilitate the transition to "budding growth."

Based on the morphological comparisons, regulatory networks, and mutant phenotypes outlined above, our conclusion is that the following hypothesis provides a reasonable framework for understanding the key developmental transition points that could lead to evolution of "aspergillioid" conidiophore from a "penicillioid" ancestor. First, the elimination of septa from the stalk could be achieved by suppressing NimX (CDK1) activity via AnkA. Second, the terminal vesicle could presumably be generated if the tip of the stalk were depolarized by inactivation of the GTPase Cdc42. This could be accomplished by upregulating the GAP RgaA, though downregulation of the Cdc42 GDP-GTP exchange factor Cdc24 might also contribute to inhibition of Cdc42 activity (Fig. 4). Third, the synchronous emergence of buds (i.e., metulae or phialides) from the terminal vesicle could require a burst of Cdc42 activity that must be shut-off (via RgaA) to prevent continued hyphal growth. A morphogenetic checkpoint analogous to that which operates in the yeast *S. cerevisiae* might provide a mechanism to couple the burst of Cdc42 activity with the cell cycle to ensure that each bud possesses only a single nucleus.[23] Finally, the timing of phialide differentiation could determine whether a particular *Aspergillus* species produces uniserate or biserate conidiophores. Because AbaA appears to specify phialide identity,[14] this step could be determined solely through the temporal regulation of *abaA* expression. Each of the transition points proposed in this hypothesis could arise by the evolution of regulatory circuits that drive changes in the temporal and/or spatial expression patterns of genes such as *ankA* or *rgaA*. For example, this could occur by recruiting these genes as targets for regulation by one or more of the transcription factors that compose the UDAs→CDP regulatory network.

The postgenomic era has ushered in an array of sophisticated tools that have significantly influenced the fields of animal and plant evo-devo by facilitating comparative genomic studies. These tools are now being applied in fungi as well, and offer considerable promise for testing hypotheses such as the one outlined above for the evolution of conidiophore development in the aspergilli. For example, the use of transcriptional profiling for comparative expression analyses has yielded important insight into limb development in animals.[24] A similar strategy could be used to compare gene expression during conidiophore development in *A. nidulans* to that of

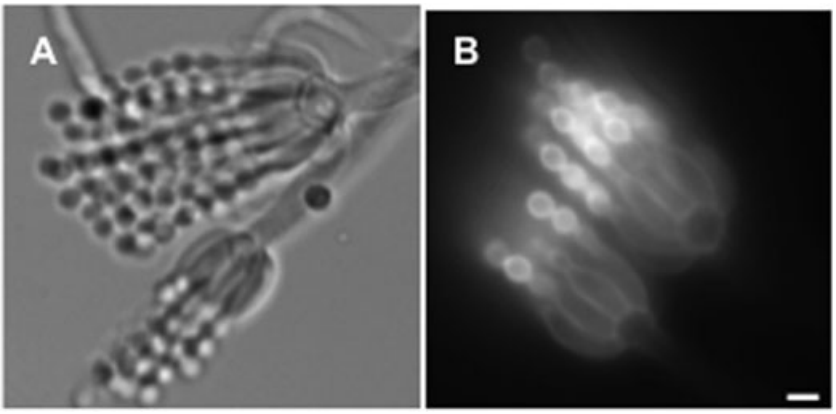

Figure 5. Conidiophores produced by *Hemicarpentales paradoxus* strain NRRL 2162. Samples in panel B are stained with calcofluor white. Bar, 3 μm.

P. chyrsogenum. Such an approach would require the ability to synchronously stage development across both species using specific events such as phialide formation as benchmarks. Another approach would be to compile transcriptional profiles for mutants defective in each component of the UDAs→CDP regulatory network. This would begin to establish regulatory links between individual transcription factors and specific cell cycle or morphogenetic events that underlie conidiophore development. Comparisons of these "regulons" across other *Aspergillus* or *Penicillium* species would then shed light on how these regulatory links might have evolved. A final approach could exploit the availability of unusual species whose conidiophore architecture is not concordant with their phylogenetic placement.[6] As an example, *Hemicarpentales paradoxus* belongs to the *Penicillium* subgenus *Penicillium* (the new name *P. paradoxus* will be proposed[6]), yet produces conidiophores with "aspergillioid" features that include an apparent terminal vesicle (Fig. 5). Assembling the genome sequence of this species would enable studies aimed at determining the molecular basis for this difference. Natural mutations or variation of this type have been extremely informative in studies of animal and plant evo-devo.

Why study the evolution of conidiophore development in the Trichocomaceae or other ascomycetes? One compelling reason is that development culminates in the production of conidiospores, which are the primary means by which many important fungal pathogens of animals and plants spread to the host. Accordingly, any information gleaned from the study of conidiophore development offers the potential to identify novel control strategies that minimize spore production and thereby reduce dissemination. However, a more immediate benefit would be to provide the first clues into how development and morphogenesis have evolved in the fungal kingdom. Although fungi are capable of developing complex multicellular reproductive structures, it is not clear whether the evolution of the underlying developmental pathways follows the same principles that have emerged from animals and plants.[1] Do regulatory networks consist of sets of genes that share common *cis*-acting regulatory sequences? Have regulatory networks been rewired by gains or losses of these regulatory sequences? Are there significant changes in the amino acid sequences of key regulators that affect how pathways have evolved? The answers to these questions will hopefully emerge in the coming years as fungal researchers begin to view development and morphogenesis from an evolutionary perspective.

Acknowledgments

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Conflicts of interest

The author declares no conflicts of interest.

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