COMMENTARY

# Mushroom functional genomics springs up

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Mushroom science and its most broadly applied aspect, the production of edible and medicinal mushrooms, has traditionally synthesized knowledge from developmental biology, genetics, studies of fungal metabolism and a great deal of empirical knowledge accumulated over the centuries of practice in mushroom production. With the advent of high-throughput -omics, the field has quickly embraced new technologies, including genome and transcriptome sequencing or proteomics. However, important stepping stones - both in terms of technology and knowledge - for linking the genes and gene expression patterns to phenotypes, have been missing. A handful of studies, all published recently, forecast a change in this respect and portrays mushroom developmental biology coming of age.

Genomic and transcriptomic resources have, as of today, been generated for many of the important mushroom-forming species in the Agaricomycetes, with a focus on fruiting body development and wood-decay, among others, and overall yielded information on the gene repertoires of most major species utilized by the mushroom industry, as well as related model- and non-model species (reviewed recently[1] ). However, both approaches have their limitations in revealing precise gene function. Since a considerable portion of research on mushroom-forming fungi focuses on fruiting body development, which is a developmental process involving the temporal and spatial coordination of cellular events, understanding patterns of gene expression regulation and revealing the precise function of genes is key. This in turn requires forward/reverse genetics and functional genomics approaches (Figure 1), a field where a recent wave of papers have made significant progress.

In a paper published recently in *mBio*,[2] identified the first cellulose-degradation related transcription factor in the Basidiomycota, Roc1 of *Schizophyllum commune*. They identified it by comparing RNA-Seq profiles of wood-grown and cellulose-grown cultures and found that the gene is conserved in the Agaricomycetes (although its possibly even more conserved and orthologous to ACE3 from *Trichoderma reesei*). Given the early emergence of Roc1, its origin may precede the emergence of efficient wood decay systems some ~300 million years ago. The *roc1* knockout mutant showed highly reduced growth on cellulose (Avicel), cellobiose and xylan, but not on other carbon sources, suggesting that Roc1 is regulating genes involved in the utilization of these carbon sources. Indeed, several CAZyme genes were no longer upregulated when the mutant was grown on cellulose and ChIP-Seq analyses identified peaks associated with several CAZyme-encoding genes such as lytic polysaccharide monooxygenases, GH3 and GH5, which are typically associated with cellulose degradation.

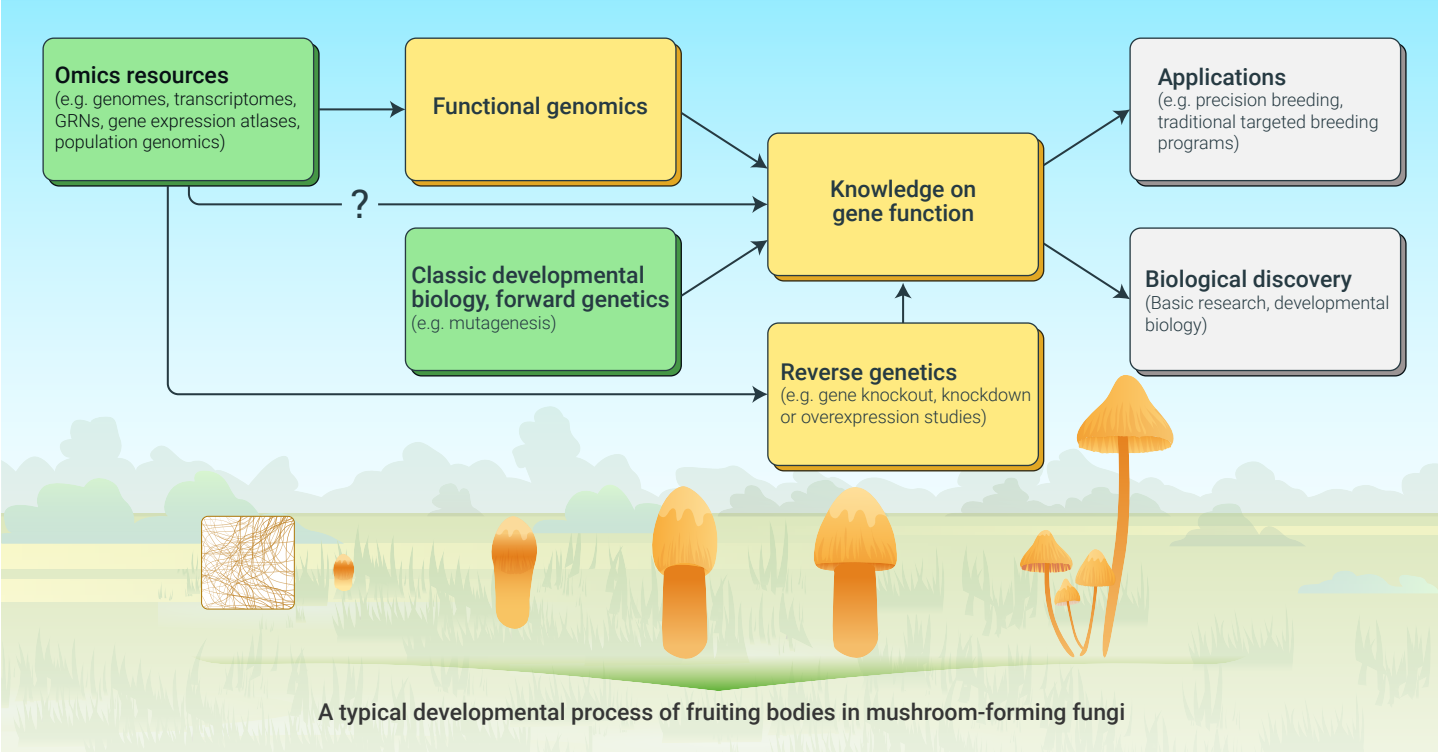


**Figure 1. The potential of functional genomics to change the current landscape of information availability in the field of mushroom science** In this paper I argue that there are plenty of omics resources, while the bottleneck to biological discovery and improved applications is the lack of knowledge on gene function. Functional genomics and reverse genetics are the best current sources of precise knowledge on gene function. On the other hand, it is questionable whether -omics studies can generate direct information on gene function. Sources of information for gene function are colored according to the Author's subjective assessment of current status. Green and yellow represent abundance and paucity of information, respectively.

The discovery of Roc1 yielded the first transcription factor involved in lignocellulose degradation in the Basidiomycota. While the regulation of this process is quite well-known in the Ascomycota,[3] the ascomycete genes are not conserved outside the phylum and basidiomycete counterparts have remained elusive so far. While there are certainly several other TFs involved in lignocellulose degradation beyond Roc1, its discovery is a significant step forward and demonstrates how functional genomics assays, including RNA-Seq, reverse genetics, ChIP-Seq and promoter analyses can now be applied

to model mushroom species such as *S. commune*, to facilitate the precise documentation of gene function.

Another recent study applied advanced functional -omics assays to map light responsive gene regulatory networks in the model mushroom *Coprinopsis cinerea*.[4] Liu et al investigated the role of CcNsdD1 and CcNsdD2 - homologs of *Aspergillus nidulans* NsdD transcription factor - in the formation of hyphal knots and photomorphogenesis using RNAi knockdown, gene expression profiling and Chip-Seq. While single knockdown mutants had no detectable phenotypes, double knockdown mutants could not produce secondary hyphal knots, their primary hyphal knots rather developed into sclerotia, which is characteristic of the wild type under constant darkness. In fact, several previously reported light-controlled genes, which are upregulated in secondary hyphal knots, were not upregulated in the mutant in response to light. This provides gene expression evidence for the lack of secondary hyphal knots in these mutants. Whether this is because the double knockdown strains were unresponsive to light, which is required for primary hyphal knots to develop into secondary, or because the mutants had a defect in a morphogenetic process is unknown. The Authors further performed ChIP-Seq analysis to identify direct target genes of NsdD2 - this analysis identified 3,741 potential target genes, a relatively high number for a specific transcription factor in the GATA family, which suggests direct interaction of NsdD2 with a large fraction of the genomes, including several light-controlled and developmental genes.

These papers made important progress in functional genomics of mushroom-forming fungi, which complements an already large body of literature on genomics and transcriptomics of these organisms, many of which would also be worth discussing. Yet, there is still a pressing shortage of knowledge on gene function in mushroom-forming fungi, especially in the context of developmental genes. Following a strong history of classic developmental biology up to the 1990's, mushroom biology virtually skipped forward to high-throughput -omics in the late 2000's, leaving important forward and reverse genetics efforts sporadic, possibly due to the recalcitrance of mushroom-forming fungi to genetic modifications, and left knowledge on gene function very patchy. In comparison, the developmental genetics and gene functions are well-known in several filamentous Ascomycota, such as *Neurospora crassa*, *Aspergillus* spp., where decades of systematic gene knockout efforts support current -omics research in uncovering gene function and key biological processes. To mitigate the shortage of knowledge on agaricomycete gene function, efforts of systematically identifying developmental genes by comparative transcriptomic atlases,[5] population genomics or epigenomics and annotating them manually[1] have come to pace recently. However, these results remain hypotheses until they are tested by functional genomics, reverse genetics or other approaches. As promising developments, several protocols for efficient genome editing by CRISPR/Cas9 have recently been reported in multiple industrial and model species, including *Coprinopsis cinerea, Schizophyllum commune, Pleurotus ostreatus* or *Ganoderma lucidum*, among others. In the future mushroom biology may step forward to utilizing global approaches such a high-throughput CRISPR screens or inferring large-scale networks, generating multi -omics resources (e.g. proteomics and metabolomics) or single-cell biology, among others, which are already coming forth in livestock animals and crop plants.

Where is mushroom science heading? Functional genomics and genome editing in mushroom-forming fungi remains one of the hardest among all fungi, but is undoubtedly needed to break through the limitations of classic -omics studies, which merely generate functional hypotheses but cannot test them mechanistically. Nevertheless, developments in this field, including the adaptation of genome editing techniques or ChIP-Seq to model species, combined with continued generation of key data resources, such as broad gene expression atlases and genomes, and will undoubtedly allow this field to grow as fast as mushrooms themselves.

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## DECLARATION OF INTERESTS

The author declare no competing interests.