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Review

Genomics and transcriptomics to study fruiting body development: An update

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ABSTRACT

Fruiting bodies of asco- and basidiomycetes are complex three-dimensional structures that protect and disperse the sexual spores. Their differentiation requires the concerted action of many genes, therefore "omics" techniques to analyze fungal genomes and gene expression at a genome-wide level provide excellent means to gain insights into this differentiation process. This review summarizes some recent examples of the use of "omics" techniques to study fruiting body morphogenesis. These include genome-centered analyses, and studies to analyze the regulation of gene expression including the analysis of RNA editing as a novel layer in the regulation of gene expression during fruiting body development in ascomycetes.

1. Introduction

The fruiting bodies of asco- and basidiomycetes are arguably the most complex multicellular structures that are produced by fungi. Fruiting bodies are the places for differentiation of sexual spores, and the fruiting body structures surrounding the spores aid in their protection and dispersal (Kües and Liu, 2000, Pöggeler *et al.*, 2006). Even though many molecular principles of the spatio-temporal regulation of fruiting body development remain to be discovered, much progress has been made in recent years, aided to a large degree by the application of "omics" techniques. This progress was reviewed some years ago (Nowrousian, 2014), but since then, a number of studies have been published using "omics" methods to gain further insights into the development of complex multicellular structures in fungi. Therefore, this review will give an update on some recent examples in this area of research. For an overview of "omics" analyses of other aspects of the biology of filamentous fungi, the reader is referred to recent reviews covering different groups of species and fungal life styles (e.g. Kazan and Gardiner, 2017, Motaung *et al.*, 2017, Muszkieta *et al.*, 2013, Toh and Perlin, 2016, Wollenberg and Schirawski, 2014).

2. Genome-centered studies and "omics"-related resources

Since the advent of next-generation sequencing techniques, *de novo* sequencing of eukaryotic genomes is no longer

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restricted to few model organisms, but is feasible for a wide range of species and can be executed even by small research groups (Nowrousian, 2010). For filamentous fungi, among the first large-scale sequencing projects were the Broad Institute Fungal Genome Initiative as well as several fungal genome projects at the MIPS (Munich Information Center for Protein Sequences); however, many of the original databases are no longer actively maintained (see below). The 1000 Fungal Genomes Project (http://1000.fungalgenomes.org), a collaboration of an international research team with the JGI (Joint Genome Institute), is currently ongoing (Grigoriev *et al.*, 2014). The aim of this project is to sequence at least two reference genomes from each of the more than 500 recognized families of fungi. The genome sequences and annotations are distributed to the research community through the JGI MycoCosm database, together with previously sequenced, publicly available fungal genome sequences. MycoCosm already holds more than 800 fungal genomes (http://genome.jgi.doe.gov/fungi/fungi.info.html). Smaller genome databases have been established for selected groups of species (e.g. Dang *et al.*, 2015, Kuan *et al.*, 2016). Fungal genome databases will greatly aid future studies of fruiting body differentiation. For example, phylogenomics studies and the identification of orthologous genes and gene families will facilitate the analysis of evolutionary trajectories of gene expression and gene function at different developmental stages (Stajich, 2017, Gabaldón and Koonin, 2013). Comparative approaches are dependent on accurate gene models, and algorithms for gene annotation and functional predictions based on transcriptome data as well as comparative genomics are under constant development (e.g. Testa *et al.*, 2015, Umemura *et al.*, 2015, van der Burgt *et al.*, 2014).

A question related to the increased number of available genomes is how to provide stable access to the research community. Maintaining and improving databases and corresponding web sites requires funding, which is usually available only for finite amounts of time, as the recent shutdown of websites for *Aspergillus* and *Neurospora* genomic resources has made painfully plain to many researchers relying on these resources (Momany, 2016). Fortunately, these and many other species are now hosted by FungiDB, which not only provides genome data, but can also hold, for example, information about gene expression and mutant phenotypes where available (Stajich *et al.*, 2012). It is possible for users to add knowledge about individual genes (e.g. about phenotypes, publications) in the form of user comments, and as manual annotation is still the best, but also among the most time- and therefore cost-intensive parts of data curation, researchers are encouraged to make use of this option to enrich the data available to the community.

With respect to fruiting body formation, *de novo* sequencing and comparative analysis of genomes can yield insights into the evolution of complex multicellular structures. This aspect of genomics was recently demonstrated in an analysis of the genome of the Taphrinomycete *Neolecta irregularis* (Nguyen *et al.*, 2017). The Taphrinomycetes are an early-branching group of ascomycetes, and while most Taphrinomycetes grow as unicellular yeasts, several genera have evolved complex multicellular structures, which in the case of *Neolecta* are reproductive structures resembling the fruiting bodies of filamentous ascomycetes (Pezizomycotina). Surprisingly, the 14.5 Mb *Neolecta* genome harbors only about 5500 genes, and therefore is more similar to the genomes of ascomycete yeasts than to the more complex filamentous ascomycetes with their larger and more gene-rich genomes. However, with respect to gene content and conservation, *N. irregularis* differs from the yeasts in that its genome contains homologs of about 1,000 genes that are lost or highly diverged in the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* (Nguyen *et al.*, 2017). In contrast, genes involved in hyphal fusion and hyphal pore gating are not generally conserved in *N. irregularis*, indicating that the evolution of complex multicellularity might have occurred independently in the *Neolecta* and filamentous ascomycete lineages, based on a core set of genes that could be recruited to this process (Nagy, 2017, Nguyen *et al.*, 2017).

Another application of genome sequencing is the identification of causative mutations in mutants generated by non-targeted mutagenesis, e.g. chemical or radiation-based mutagenesis approaches. Recent examples of sequenced mutants with defects in fruiting body formation include the identification of the novel developmental gene *spd4* in *Sordaria macrospora* (Teichert *et al.*, 2017b), and components of NOX (NADPH oxidase) complexes in *S. macrospora* and *P. anserina* (Dirschnabel *et al.*, 2014, Lacaze *et al.*, 2015). The *noxD* gene identified in *P. anserina* encodes a homolog of the mammalian p22phox protein, a member of NOX complexes in mammals for which no fungal homolog had been identified before (Aguirre and Lambeth, 2010, Marschall and Tudzynski, 2016). Its function in fruiting body formation might be conserved as the corresponding *S. macrospora* mutant is sterile, and a *Botrytis cinerea* mutant is unable to produce sclerotia, from which the fruiting bodies of *B. cinerea* are generated (Nowrousian *et al.*, 2007, Siegmund *et al.*, 2015).

The availability of genome sequences has enabled large-scale projects to generate deletion mutants in several fungi. With respect to fruiting body formation, especially large-scale knockout studies in *Neurospora crassa* and *Fusarium graminearum* have been informative. The first large-scale deletion analysis of about 100 transcription factor genes of *N. crassa* was published in 2006, and a recent study complemented the first by analyzing 273 viable deletion mutants of the predicted 312 transcription factor genes of this species (Carrillo *et al.*, 2017, Colot *et al.*, 2006). *F. graminearum* contains more than twice the number of transcription factor genes than *N. crassa*, and the corresponding deletion mutants were studied in a large-scale knockout project (Son *et al.*, 2011). In *N. crassa*, mutants in genes encoding catalytic subunits of serine/threonine and tyrosine protein phosphatases as well as G protein-coupled receptors were also analyzed already (Cabrera *et al.*, 2015, Ghosh *et al.*, 2014). In all studies, sexual development was one of the phenotypes that were scored during the analysis of mutant phenotypes. The resulting mutant strains that deviate from the wild-type phenotype with respect to fruiting body development make excellent candidates for in-depth studies of the molecular functions of the corresponding genes.

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