Comparative genomics reveals the origin of fungal hyphae and multicellularity

Ph.D. Dissertation

Enikő Kiss

Supervisor: László Nagy Ph.D.

Synthetic and Systems Biology Unit

Institute of Biochemistry

Biological Research Centre, Szeged

Doctoral School of Biology, Faculty of Natural Sciences and Informatics

University of Szeged

Szeged

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List of abbreviations

ADP - adenosine diphosphate
APSES - ASM-1, PHD1, STUA, EFG1, and SOK2 proteins
ASR – ancestral character state reconstruction
ATP - adenosine triphosphate
AVC - apical vesicle crescent
BCZ – Blastocladiomycota, Chytridiomycota, Zygomycota
cAMP - cyclic adenosine monophosphate
CAR - contractile actin ring
CAZy - carbohydrate active enzyme
CDK - cyclin-dependent kinases
CDS - coding sequence
CELSRs - cadherin EGF LAG seven-pass G-type receptors
CTK - cytoplasmic tyrosine kinases
CWI - cell wall integrity
DNA - deoxyribonucleic acid
DOCK - dedicator of cytokinesis
ECM - ectomycorrhiza
ECM - extracellular matrix
EE - early endosome
ELMO - engulfment and cell motility
FDR - false discovery rate
GDP/GTP - guanine di/tri nucleotide
GEF - guanine nucleotide exchange factor
GPCR – G-protein-coupled receptor
GPI – glycosylphosphatidylinositol
GTP - guanosine triphosphate
HHK – hybrid histidine kinase
HOG - high-osmolarity glycerol
HRGP - hydroxyproline-rich glycoprotein
IPR - InterPro
LCA - last common ancestor
LG – matrix called LG after Le and Gascuel

MAPK - mitogen-activated protein kinase
MC – multicellularity
MCL - Markov Cluster
MCMC - Markov chain Monte Carlo
ML – maximum likelihood
MRCA - most recent common ancestor
MT - microtubule
NADPH - nicotinamide adenine dinucleotide phosphate
PI(4,5)P2 - phosphatidylinositol 4,5-bisphosphate
PKA - protein kinase a
PP - posterior probability
PTK - protein tyrosine kinases
RAM - regulation of ACE2 and morphogenesis
RGS - regulator of G protein signaling
RTK - receptor tyrosine kinases
SIN - septation initiation network
SNARE - SNAP receptor
SPK - Spitzenkörper
SPS - SSY1–PTR3–SSY5
TF - transcription factors
TKL - tyrosine kinase-like
TORC - target of rapamycin complex
TPM - tropomyosin
UC - unicellular
UNICORN - unicellular opisthokont research initiative
WAML - WASP and MIM like
WASH - Wiskott–Aldrich syndrome protein and SCAR homologue
WASP - Wiskott-Aldrich syndrome proteins
WAVE - Wiskott-Aldrich syndrome protein family verprolin homologs
WAWH - WASP without WH1 domain
WGD – whole genome duplication
WHAMM - WASP homolog-associated protein with actin, membranes and microtubules
WOPR - novel DNA-binding domain in WOR1 TF

„What then new had to be “invented” for the transition from single-cell organisms to multicellularity? What properties did these so-called simple organisms have, which were and are still fundamental for the development of multicellular organisms? How did embryonic development from an egg evolve? The key features required for embryonic development are a program of gene activity, cell differentiation, signal transduction so cells can communicate with each other, and cell motility to generate change in form. Single-cell organisms possessed all of these features in a primitive form and thus very little needed to be invented, although much had to evolve and be fine-tuned.”

“Is then the evolutionary transition to multicellularity a difficult one or not? The blunt answer is, not at all, since multicellularity has arisen more than twenty times in evolution. However, there are only three lineages that produced complex organisms: plants, animals, and fungi. Three hits in 3.5 billion years are not that many.”

Szathmáry & Wolpert (Genetic and Cultural Evolution of Cooperation, 2003, p. 301)

1. Introduction

1.1. Evolution of multicellularity

1.1.1. Transitions to multicellular life

Early in Earth's history organisms were single-celled. Multicellularity arose subsequently and evolved independently from unicellular ancestors in ten different major clades and over 25 times in individual lineages (Figure 1.) (Bonner, 1998, 2009; Buss, 1987; Carroll, 2001; Kaiser, 2001; King, 2004; Kirk, 1998; Knoll, 2011; Medina et al., 2003; Rokas, 2008; Shapiro, 1998; Szathmáry & Smith, 1995). The evolution of multicellularity is considered one of the major transitions in the history of life with the first evidences dating back to 3-3.5 billion years (Knoll et al., 2006). Certain lineages got more and more complex during evolution, and one of the biggest questions of life is why and how this complexity arose and increased in different species (Szathmáry & Smith, 1995).

Figure 1. Phylogenetic tree of major clades where multicellularity evolved during evolution. Groups representing complex multicellular organisms are labelled with asterisk. (Figure is from Brunet et al., 2017).

The formation of higher-level units from lower-level ones can be the result of different kinds of evolutionary innovations. Each prokaryote and eukaryote lineage where multicellularity has evolved represents a unique solution to the challenges of multicellular organization, as a result of diverse selection and the genomic environment (Sebé-Pedrós et al., 2017).

Based on differences in complexity levels, two major types of *multicellularity* (MC) can be distinguished, simple and complex multicellularity. Simple multicellular organisms form filaments, cell clusters, balls and sheets of cells, where almost every cell is in direct contact with the environment. Adhesion is present in these simple multicellular forms, but the intercellular communication and transfer of resources are very limited. Complex multicellular forms, however, show more sophisticated cell adhesion, intercellular communication, bulk transport and programmed cell death, which are indispensable for cell- and tissue-differentiation and also for complex multicellular development. In contrast to simple multicellular organisms, complex bodies display three-dimensional structures and as a result of this complexity, only outer cells are in contact with the environment. For both simple and complex multicellular organisms, there are two ways to become multicellular, namely the clonal and aggregative development (Figure 2.) (Brown et al., 2009; Brunet & King, 2017; Glöckner et al., 2016; Sebé-Pedrós et al., 2013, 2017). Multicellular bodies can develop via mitotic division of a progenitor cell, where the resulted cells stay together (clonal multicellularity) (Michod et al., 2003; Koenig & Nedelcu., 2016). Or another way, where cells disperse after cell division and come together later to form multicellular bodies (aggregative multicellularity) (Du et al., 2015). These routes are differ in how multi-celled precursors emerged by adhesion, cooperation, communication and functional diversification of cells (Abedin & King, 2010; Fairclough et al., 2013; Ruiz-Trillo et al., 2007). The general assumption is that the genetic uniformity of cells in clonal multicellularity leads to higher evolutionary stability and success than in aggregative multicellularity, which is in good accordance with the fact that the most diverse multicellular lineages (e.g. Plants, Metazoa) have evolved through clonal intermediates (Brunet & King, 2017; Pentz et al., 2018; Sebé-Pedrós et al., 2017).

Figure 2. Schematic representation of clonal (A) and aggregative (B) routes of multicellular development.

Besides lineage-specific, unique solutions, which resulted in a great morphological diversity, the formation of multicellular structures lies on same bases in different eukaryote lineages. These solutions are the size advantage, programmed cell division and the differentiation of soma and the early-isolated germ line. These preconditions later opened the door for the evolution of five core innovations, such as cell adhesion, intercellular communication, cell- and tissue-differentiation and the division of labor, that facilitated the transition to multicellular organisms from unicellular ancestors (Szathmáry, 2015). Establishing a stable cell adhesion, a strong attachment between cell surfaces after cell division, was a key mechanism to build multicellular body structures. The establishment and maintenance of these cellular connections followed different routes in different organisms, but it served the same purpose everywhere, that is to strengthen the structural integrity (Abedin & King, 2010). In volvocine algae, the evolution of the extracellular matrix (ECM) enabled the creation of stable attachments between cells, whereas the same role was fulfilled by the plasmodesmata (cytoplasmatic channels that connect neighboring plant cells to each other) and different types of junctions (e.g. gap junction, tight junction, etc.) in plants and animals, respectively. Attaching neighboring cells together allowed organisms to evolve more sophisticated intercellular interaction networks. Fine tuning of signaling and the evolution of protein-protein interaction networks resulted in highly efficient intercellular communication systems. These systems were indispensable for both the evolution of intercellular cooperation and the division of labor, where differentiated cells lose their autonomy and become cooperating partners. This intercellular collaboration in different morphogenetic mechanisms opened the door for creating complex multicellular body structures (Kirk, 2005).

The size-complexity rule (Bonner, 2004) explains how the multicellular body size-increase could require an increase in complexity, division of labor among cells, and also in the number of cell types. The selection for size-increase over geological time enabled multicellular organisms to capture and convert energy from food in a much more efficient way for their different living processes (Bonner, 1965). The aforementioned inventions enabled the emergence of complex multicellular traits, like respiratory systems, nutrient transport systems, tissues and organs. The size-increase offered great advantages for animals in gaining mobility, seeking better habitat, escaping from predators and chasing preys (Berleman & Kirby, 2007; Boraas et al., 1998; Herron et al., 2019). The increased size and the invention of wood of land plants, for example, gave the strength to support

masses and to maximize photosynthesis. Also, the deeply invading roots enabled a much more efficient uptake of water and nutrients. Saprotrophic and parasite fungi on the other hand took the advantage of size increase to built reproductive structures and spread their spores more efficiently. Over a long period of time, there has been an overall increase in body size, and becoming a multicellular organism was an easy way of accomplishing this size-increase (Bonner, 1998).

These different evolutionary inventions (adhesion, cell-to-cell communication, division of labor between cells) leading to the emergence of multicellularity enabled organisms to be more efficient under certain conditions, and thereby created an enormous evolutionary advantage for them in different ecological niches (Bonner, 1998).

1.1.2. Genome sequencing for understanding the evolution of multicellularity

Researches of the last few years have revealed that the evolution of multicellular development is associated with genes involved in cell adhesion, cell-to-cell communication and cell differentiation (Knoll, 2011; Rokas, 2008). Also, it was discovered, that the different components of the genetic toolkit responsible for multicellular development originated and diversified at different time points during evolution (Abedin & King, 2008; King & Carroll, 2001; King, 2004; King et al., 2003; Larroux et al., 2008; Nichols et al., 2006; Rokas, 2008). These investigations could not have happened without the extensive genome sequencing projects and the analyses of several non-model organisms. Performing comparative genomic studies on the newly sequenced genomes of unicellular protists (choanoflagellates, filastereans, ichthyosporeans), which are the closest relatives of metazoans and fungi, was crucial for the investigation of the origin and evolution of multicellularity. The UNICORN (unicellular opisthokont research initiative) project performed extensive genome sequencing on the closest unicellular relatives of animals and fungi and provided an extremely powerful dataset to investigate the genetic background of multicellularity. Notable examples of whole genome-sequenced species are the followings: *Capsaspora owczarzaki*, *Monosiga brevicollis*, *Sphaeroforma arctica*, *Creolimax fragrantissima*, *Amoebidium parasiticum*, *Nuclearia simplex*, *Allomyces macrogynus*, *Spizellomyces punctatus*, *Fonticula alba*, among others (Ruiz-Trillo et al., 2007).

1.1.3. Multicellularity-related genes and functions

1.1.3.1. Evolution of adhesion-related genes

One of the main prerequisites of the evolution of multicellularity is the stable cell-to-cell attachment. Cell adhesion mechanisms evolved in different ways during evolution, and achieved by the production of various structures – extracellular matrix (ECM), cell-to-cell junctions, pectinaceous middle lamella and plasmodesmata – in the different phylogenetic groups (Figure 3.) (Daher & Braybrook, 2015; Lucas et al., 2009; Ordaz-Ortiz et al., 2009).

Figure 3. Cell adhesion structures in different phylogenetic groups. (A) Extracellular matrix of *Volvox* species. (B) Pectinaceous lamella and plasmodesmata of plant cells. (C) Cell-to-cell junctions in animals.

Major gene families (integrins, cadherins, lectins, collagens, laminins, fibronectins and immunoglobulins) mediate these processes. Not only the cell adhesion but also cellular morphogenesis and cell polarization rely on these gene families (Cereijido et al., 2004; King et al., 2003). Comparative genomic studies on multicellular organisms and their unicellular ancestors, and the investigation of the evolutionary history of these adhesion-related protein families revealed how cell adhesion mechanisms evolved in different phylogenetic groups during evolution (Abedin & King, 2008, 2010). These analyses highlighted that certain adhesion-related gene families are already present in the unicellular relatives of metazoans and represent a pre-adaptation for adhesion. For example, the hydroxyproline-rich glycoprotein (HRGP) cell wall component family were expanded and diversified during evolution, and co-opted/exapted for different cellular functions in multicellular organisms. For example, the mechanism of co-option opened the door for evolving the ECM in *Volvox* species (Abedin & King, 2010). Co-option/exaptation is a term used, when a function or trait changes during evolution. It happens, when a useful character is not shaped by natural selection for its current role,

rather the feature, which was previously evolved for a particular function, later serves similar (co-option) or completely different functions (exaptation) under different conditions (Gould & Vrba, 1982, McLennan, 2008). Furthermore, the comparison of cadherins in *Monosiga brevicollis* and its metazoan sister taxa revealed, that in metazoan progenitors the cadherins possibly fulfilled their roles as receptors for capturing extracellular signals, and were co-opted for serving as adhesion receptors in epithelial cells to mediate cell-to-cell interactions in multicellular metazoans (Abedin & King, 2008, 2010). Also, the investigation of integrins in *Capsaspora owczarzaki*, which is an unicellular relative of metazoans, revealed that integrin functions co-opted for establishing animal multicellularity, possibly playing a role in substrate attachment in animal ancestors (Abedin & King, 2010).

1.1.3.2. Evolution of signalization networks related to multicellular development

The evolution of transmembrane receptors and the diversification of signaling molecules enabled cells to capture signals from the environment that was not possible previously. Thanks to these innovations, the signaling networks could mediate a more efficient cell-to-cell communication and regulation, allowing the evolution of multicellular structures. The diversification of signaling molecules and downstream effectors (e.g. transcription factors (TFs)), also the more sophisticated spatial and temporal control of the major signaling networks were fundamental for key functional innovations in complex multicellular lineages (Loh et al., 2016; Marijuán et al., 2013; Umen, 2014). Investigation of the evolutionary history of 71 transcription factor classes revealed, that the most complex organisms have the most complex transcription factor networks (Mendoza et al., 2013). In Metazoa and Dikarya, where complex multicellular species emerged, the ancestral TF toolkit has changed during evolution, and a genome-wide transcriptional rewiring has occurred. It was also observed, that Metazoa and Embryophyta have the richest transcription factor repertoires comparing to other eukaryotes (Mendoza et al., 2013). In these two clades, a two-step expansion of the Homeobox, bHLH and bZIP repertoires was found, first in their unicellular ancestors, and later at the origin of Metazoa and Embryophyta (Mendoza et al., 2013). The expressional patterns of these transcription factors also changed during development, and these changes depended on the evolutionary age of the corresponding TF. It is assumed that these differences between eukaryotic lineages might be partly due to the evolution of complex embryonic

development in Metazoa and Embryophyta (Mendoza et al., 2013). The investigation of the evolution of G-protein-coupled receptor (GPCR) signaling system also highlighted interesting evolutionary changes related to multicellularity. The GPCR signaling system has a role in the growth control and migration of cells, and sensing environmental conditions. Investigation of GPCR signaling system components in different phylogenetic groups revealed that the cytoplasmic elements of the system are quite conserved – in respect of their presence and gene copy numbers – across both unicellular and multicellular species, as opposed to transmembrane receptors that showed a big copy-number expansion in Metazoa, compared to its unicellular sister clades (de Mendoza et al., 2014). Similar pattern was found during the investigation of other signaling pathways. The Notch signaling pathway regulates development and cell differentiation. Investigation of this pathway in 35 species revealed that this signaling system evolved partially by the co-option/exaptation of ancient pan-eukaryotic genes, but also by the recruitment of newly evolved proteins (Notch, Delta) and protein domains (MNLL, DSL) that emerged in metazoans. These novel protein components were responsible for the fine tuning of the ancient signalization network and increasing its complexity in eumetazoan lineages (Gazave et al., 2009). Another well-investigated network is the Hippo signaling network that regulates cell proliferation and maintains the strict control of cell number and the size of organs (Halder & Johnson, 2011). Components of this pathway are already present in unicellular ancestors of Metazoa, and the original function of this signaling network is assumed to regulate cell polarity in order to help cell-substrate adhesion and to regulate cell proliferation as well in response to cell density. The co-option of these functions enabled the different metazoan lineages to develop a strictly controlled proliferation machinery, serving as an essential contributor to the transition to metazoan multicellularity (Sebé-Pedrós et al., 2012).

1.1.3.3. Evolution of multicellularity-related genes in fungi

The vast majority of the studies investigating multicellularity focuses mainly on the evolution of metazoans. Huge amount of data was produced on metazoan multicellularity, but we have limited understanding on the evolution of fungal multicellularity. Genomic investigation of the *Coprinopsis cinerea*, and its comparison to other fungal genomes revealed that Agaricomycotina genomes display large expansion of the gene families of

P450s (Nelson, 2013; Omura, 2013) and hydrophobins (Nagy et al, 2018), both functioning as key contributor to multicellular life. The expansion of the protein kinase FunK1 gene family was also observed in the *Coprinopsis cinerea* genome, which family was found only in multicellular fungi and possibly plays a role in developmental processes specific to multicellular fungi (Stajich et al., 2010). The expansion of Homeobox, bHLH and bZIP transcription factor repertoires was also investigated in 16 fungal species and other 61 eukaryotes (Mendoza et al., 2013). Based on this study, fungi showed a less complex transcription factor repertoire (TFome) compared to Metazoa and Embryophyta, and the TFome profile of fungi was highly specific with many fungal-specific TFs. Gains of new transcription factors were not common in fungal species – as opposed to Metazoa and Embryophyta - except STE-like and Copper-fist TFs. Only Dikarya, the crown group of fungi changed its ancestral TF repertoire during evolution (Mendoza et al., 2013). The transcription factor repertoires of early diverging fungi (Chytridiomycota, Blastocladiomycota, Zygomycota) are quite diverse, and resembles to unicellular relatives. This comparison of TF repertoires across 77 species (Mendoza et al., 2013) highlighted an important difference between the regulation of the modular development of fungi, and the embryonic development of Metazoa and Embryophyta, where a more complex regulation was needed. Furthermore, a recent transcriptomic study (Krizsán et al., 2019) also provided interesting details about the regulation of fungal development and the processes underlying the complex multicellularity. This study revealed the importance of various proteins in mushroom development, including cell wall-modifying enzymes, adhesion-related proteins, secreted proteins, tyrosine kinase-like (TKL) kinases and F-box transcription factors.

1.2. Gene duplications and losses as a major force in evolution

The first speculations concerning the importance of gene duplication events date back to the 1930's. J.B.S. Haldane was the first, who hypothesized that gene duplications can serve as potential buffers against deleterious mutations during evolution (Haldane, 1932). H. J. Muller found that duplicated gene copies diverge and evolve new functions due to mutations acquired over time (Muller, 1935), and A. Sturtevant and C. B. Bridges, Mullers's colleagues were the first, who discovered that Bar mutation was a duplication (Bridges, 1936, Muller, 1936). In 1970, Susumu Ohno moved forward and postulated that

gene duplication can be the major force of functional innovation and evolution (Ohno et al., 1968; Ohno, 1970). He suggested that large changes had been made during evolution, possibly by the acquisition of new gene loci and new proteins with previously non-existing functions. During the past few decades, gene duplication events had attracted more and more attention, and thanks to this intense interest, our knowledge on gene duplications is greatly expanded. Gene duplication is a type of DNA duplication that originate from different kinds of genomic rearrangements, such as homologous unequal crossing-over, nonhomologous crossing-over, copy-and-paste transposition and chromosomal/genome duplication (Graur, 2016). These rearrangements follow completely different molecular mechanisms. For example, the unequal crossing-over generates duplicated DNA segments which are linked to each other on the same chromosome. This mechanism is termed tandem gene duplication. On the other hand, in the case of copy-and-paste transposition, a transcribed complementary DNA is created from the mRNA. This DNA fragment is randomly inserted into the genome subsequently, creating a new copy that is not linked to the original one. After the DNA replication and during the chromosomal disjunction errors could lead to large-scale duplications, such as chromosomal or whole genome duplications. The duplicated gene copies are usually called as paralogs (different from orthologs, where the gene copies are the results of speciation event and not gene duplication), that share common ancestral sequence and group into multigene families. After duplication, four types of processes can determine the evolutionary fate of the duplicated copies: nonfunctionalization, conservation, neofunctionalization and subfunctionalization (Figure 4.). Firstly, in the case of nonfunctionalization, one of the duplicated copies can lose its original function in a relatively short time-frame, due to the gradual accumulation of deleterious mutations. Nonfunctionalization/pseudogenization generates not expressed and non-functional pseudogenes, which will be deleted from the genome or will become unrecognizable “copy” of the original gene. This is the most frequent and the most likely scenario of the evolution of duplicated genes. Secondly, paralogous genes can retain their original function, and they can be the subject of gene conservation, if there is a high demand for the gene products and the duplicated copies are beneficial (Lynch, 2002; Lynch & Conery, 2000). However, this gene conservation mechanism is the least likely way of the evolution of duplicated gene copies. If the extra amount of gene product is not advantageous, the duplicated copies of genes cannot be maintained in the genome and subfunctionalization happens. It can occur at the level of transcription, if the duplicated

copies show different expression patterns (Force et al., 1999), or at the level of protein function (Zhang et al., 2002). Finally, in the case of neofunctionalization, an entirely new function evolves after gene duplication. This is probably the most interesting fate of a duplicated copy from an evolutionary perspective (Zhang, 2003; Zhang et al., 1998), but is much less frequent comparing to nonfunctionalization and subfunctionalization. As a result of these mechanisms, some of the duplicated gene copies can be fixed in the genome for a long time, whereas others can be inactivated or lost from the genome. This model is known as the birth-and-death evolution model (Nei et al., 1997), which was being investigated in the case of many gene families. Also, this model is assumed to be the driving force of building new genetic systems related to multicellular development in animals (Wagner et al., 2003), plants (Nam et al., 2004), and the development of adaptive immune system (Hughes & Nei, 1989; Nei et al., 1997) among others (Nei & Rooney, 2005). For example, in vertebrate evolution, the expansion and divergence of ATP-binding cassette protein family represent a great example of this kind of gene family evolution (Annalo et al., 2006).

Figure 4. Evolutionary fate of duplicated gene copies

1.2.1. Diversification of multicellularity-related gene families

The role of gene duplication and gene family diversification in big evolutionary events is a widely investigated field of biology. Gene duplications are considered to be a prerequisite for the evolution of new structures and functions, providing genetic substance for mutations and selection, and making new changes possible in major evolutionary events, like the transition to multicellularity and complexity. The

diversification of gene families by gene duplications and partial gene duplications resulted in an enormously big functional diversity, and some of these events took place in early evolution, before the emergence of the different lineages. There are many examples of diversification events in early metazoan evolution where gene duplications played key roles in the evolution of complexity. For example, protein tyrosine kinases (PTKs) and G-protein α subunits, which are key contributors to signal transduction mechanisms showed extensive duplication and gene family divergence which predate the parazoan-eumetazoan split (Suga et al., 1997, 1999, 2008). Studies on protein tyrosine kinase repertoires in the filastereans (*Capsaspora owczarzaki*, *Nuclearia sp.* and *Fonticula alba*) revealed differences in gene expansions between cytoplasmic tyrosine kinases (CTKs) and receptor tyrosine kinases (RTKs). It was found that CTKs were already present at the common ancestor of Holozoa (Metazoa, Filasterea, Choanoflagellida, Ichthyosporea and Corallochytraea), while the RTK toolkit in the different species was quite diverse, and highly expanded by gene duplications in a clade-specific manner (Suga et al., 2012, 2014). The evolution and diversification of cadherin families was crucial for the evolution of multicellular metazoans, due to their importance in cell-to-cell adhesion, polarity establishment and morphogenesis. To investigate the evolutionary origin and diversification of cadherin genes, comparative analysis was performed on *Capsaspora*, *Salpingoeca rosetta* and the sponge *Oscarella carmela* (Nichols et al., 2012). The evolutionary reconstruction of cadherins revealed that the last common ancestor (LCA) of choanoflagellates and metazoans contained different kinds of conserved cadherin families, for example the lefhtyrin and coherin families, which may have functions specific to collar cells in sponges and choanoflagellates, and hedgling, which is restricted to choanoflagellates, sponges and cnidarians). Beside these evolutionary conserved families, two other cadherin families evolved at the origin of Metazoa, the “classical cadherin” and CELSR/flamingo families. All of the investigated families are present in the sponge *Oscarella carmela*, and it was also found that the origin of “classical cadherins” coincides with the evolution of multicellular metazoans. This “classical cadherin” family promotes cell adhesion by the interaction with β -catenin, and this interaction could serve as a key feature for the evolution of complexity and multicellularity (Nichols et al., 2012). Studies on transcription factor genes also revealed expansion in specific gene families. Comparative analysis on the sponge *Amphimedon queenslandica*, other metazoans and the choanoflagellate *Monosiga brevicollis* showed expansion in the number of transcription factor genes (Homeobox, Sox, T-box, Fox)

(Larroux et al., 2008). These TFs are members of conserved developmental networks in metazoans and diversified by gene duplication events before the separation of metazoan lineages. Also, a second expansion of TF repertoire occurred after the split of sponges and eumetazoans. This second expansion resulted in a more diverse and complex regulatory systems in Eumetazoa. This analysis on specific TFs highlights how ancient developmental programs can evolve during evolution by gene duplication events in complex metazoans (Larroux et al., 2008). But there are several other examples of evolution by gene duplications. The seven animal subtypes of cyclic nucleotide phosphodiesterases with distinct domain organization were also the results of gene duplications and divergence before the separation of sponges and animals (Koyanagi et al., 1998). Furthermore, bZIP transcription factors were also the subject of multiple independent expansions. These TFs are key regulators implicated in basic cellular functions, cell proliferation and differentiation, and their diversification was restricted to multicellular organisms. These observations are consistent with the notion that these TFs play role in complex regulatory systems that underlie cell type-specific gene regulation and expression in different multicellular lineages (Jindrich & Degnan, 2016). Additionally, SNARE proteins that regulate the docking and fusion of vesicles to target membranes, were also increased in numbers and types in different eukaryotic lineages by gene duplications and diversification, at the time of the first major evolutionary transitions. The diversification of SNAREs enabled the evolution of a complex, fine-tuned and much more efficient endosomal sorting system, and a more specialized secretory pathway system, which gave great evolutionary advantage to the first multicellular organisms, land plants and multicellular metazoans (Kloepper et al., 2008; Sanderfoot, 2007).

1.3. Gene families that predate animal origins and multicellularity

Choanoflagellates, filastereans, ichthyosporeans, multicellular metazoans and fungi share a unicellular common ancestor. Recent comparative genomic studies on multicellular species and their unicellular relatives revealed that key multicellularity-related genes – that were previously thought to be exclusive to multicellulars – were already present in their unicellular ancestors (Grau-Bove et al., 2017; King et al., 2008; Richter et al., 2018). The genome analysis of *Monosiga brevicollis* (Choanoflagellata) revealed that cadherins,

essential contributors to metazoan cell adhesion are present in its genome, despite the fact that this organism has a unicellular lifestyle and do not need cadherin-mediated adhesion. Based on this result, it was shown that cadherin genes predate the origins of metazoan multicellularity (Abedin & King, 2008). Furthermore, not only cadherins but also C-type lectins and key components for cell signaling (tyrosine kinases, G-protein-coupled receptors) are present in unicellulars and predate animal origins. These gene families evolved before the origin of metazoan multicellularity and probably co-opted for multicellularity-related functions during metazoan evolution (King et al., 2003). Further investigation of the *Monosiga brevicollis* revealed that beside adhesion and signaling-related genes, a limited repertoire of metazoan TFs (p53, Sox, Myc) was also present in its genome, but others (Hox, T-box etc) were missing, indicating that the latter are strictly metazoan-specific TFs (King et al., 2008). The evolutionary conservation of the integrin-mediated cell adhesion and signaling was also investigated by comparative genomic approach on a broad range of species. This analysis revealed that key genes related to this process originated much earlier than metazoan multicellularity (Sebé-Pedrós et al., 2010). Also, the investigation of the origin and evolution of developmental metazoan transcription factors uncovered that *Capsaspora owczarzaki*, one of the unicellular relatives of metazoans shows a highly abundant TF repertoire (Sebé-Pedrós et al., 2011). Moreover, this analysis uncovered two major TF expansions, one for bZIPs and bHLHs before the split of *Capsaspora* and Metazoa, and another for Homeodomain, Sox, bZIP and bHLH TFs during metazoan diversification. Other metazoan multicellularity-specific TFs (Runx, NF-kappaB, p53) showed deep evolutionary conservation. The emergence of these deeply conserved families happened before the divergence of *Capsaspora*, Choanoflagellata and Metazoa lineages (Sebé-Pedrós et al., 2011). Moving forward to functional genomic analyses, recent investigations on the filasterean *Capsaspora* TFome revealed that the downstream target networks of these conserved transcription factors are also highly conserved between *Capsaspora* and metazoan lineages. On the other hand, in contrast to metazoan species, the genome of *Capsaspora* showed a lack of, or only a marginal presence of long-range cis-regulatory elements and syntenic regulatory blocks that are key components of complex regulatory mechanisms playing role in multicellular metazoan development. Also, due to the small size of the cis-regulatory elements and the lack of metazoan-specific promoters in *Capsaspora* genome, the complex combinatorial binding of multiple TFs and the cell-type-specific regulation (both are characteristic to multicellular metazoans) are not possible in the unicellular *Capsaspora*. Due to these

functional differences between multicellular and unicellular lineages, it can be possible that the fine-tuning of transcription regulation networks and the increase in regulatory complexity could be the key factors to the evolution of multicellularity (Sebé-Pedrós et al., 2016, 2017).

1.3.1. „Minor Major Transition” hypothesis

For a long time, it has been assumed that the increase in complexity may have been achieved as a result of a series of major evolutionary transitions, such as the formation of chromosomes, the compartmentalization of eukaryotic cells or the evolution of multicellular organisms from unicellular ancestors (Szathmary & Smith, 1995).

However, doubts have been raising about this view, and recent studies show that the transition from unicellular to multicellular organization may not have been so hard after all, and that this transition was not as major of a genetic leap as we previously thought (Grosberg & Strathmann, 2007). Several evidences support these assumptions. Firstly, major transitions are considered to be rare evolutionary events. However, multicellularity evolved independently at least 25 times during evolution within different phylogenetic groups (Knoll, 2011). Furthermore, in *Bacillus subtilis* (Branda et al., 2001), the alga *Scenedesmus acutus* (Van Donk & Lurling, 2000), the myxobacterium *Myxococcus xanthus* (Kaiser, 2001) and in the cellular slime mold *Dictyostelium discoideum* (Bonner, 1998) this transition is relatively easy, and the development of multicellular fruiting bodies in these species is quickly inducible in response to environmental stimuli. The repeated transitions from unicellular to multicellular forms in the fungal kingdom, and the fact that complex multicellular fruiting bodies evolved about a dozen times during fungal evolution could also mean, that the hurdles accompanied with this change were not that big as we previously thought (Nagy, 2017; Nagy et al., 2018). Secondly, the conservation of multicellularity-related genes in unicellular organisms, and the discoveries that many requirements for multicellular development, such as cell-to-cell adhesion, intercellular communication, programmed cell death and developmental regulation likely existed in ancestral single celled precursors (Grau-Bové et al., 2017; King et al., 2008; Richter et al., 2018) also raise the question, whether this transition can be considered truly as a major evolutionary event. Comparative genomic studies revealed that large proportion of the deeply conserved morphogenetic toolkit associated with multicellular organization is co-opted and evolved for novel functions in

multicellulars. These evolutionary mechanisms along with certain genetic innovations (for example the emergence of metazoan-specific TF repertoire and the evolution of promoters and cis-regulatory elements) could be accounted for complex morphological changes. Based on these observations, a hypothesis was formulated that the transition from unicellular to multicellular organization might not represent a major evolutionary event but a relatively easy minor major transition (Grosberg & Strathmann, 2007; Wolpert & Szathmáry, 2002).

1.4. Fungal multicellularity

1.4.1. Unique Fungal Multicellularity

Examples of multicellular forms are present in all major eukaryotic lineages. Among the three big eukaryotic kingdoms, where multicellular forms dominate among extant species, fungi represent a markedly different route to evolve multicellularity. Whilst most multicellular lineages can be recognized as either clonal or aggregative by comparisons to their unicellular relatives (Niklas & Newman, 2013; Niklas, 2014; Sebé-Pedrós et al., 2017), fungal multicellularity might represent a third route (Nagy et al., 2018), by evolving characteristic multicellular structures, so called hyphae. These thin, tubular structures grow in a polarized fashion to form fractal-like mycelial networks and multicellular hyphal thalli. These mycelial networks have a supporting role in the invasion of substrates for nutrients by saprobes, pathogens and symbionts, and also have key roles in fungal reproduction.

Fungal multicellularity has a number of unique features compared to clonal and aggregative multicellularity, raising the possibility that its evolution follows markedly different principles (Nagy et al., 2018). In contrast to clonal and aggregative lineages, where the first multicellular bodies evolved by cell adhesion (e.g. *Salpingoeca rosetta*) (Fairclough et al., 2010) or aggregation (e.g. *Capsaspora owczarzaki*) (Sebé-Pedrós et al., 2013), filamentous hyphae might have evolved by the gradual elongation of substrate-anchoring, anucleate hypha-like outgrowths (rhizoids) of early fungal ancestors through tubular, multinucleate intermediates. These rhizoids resemble the early diverging fungal lineage Chytridiomycota (Dee et al., 2015; Harris, 2011; Laundon et al., 2019; Nagy et al., 2017; Stajich et al., 2009). In multicellular organisms the cooperation between

individual cells is beneficial for the whole organism, but it can also be costly or beneficial to the cooperators. Therefore, the evolution of multicellular organisms had to face and handle these emerging conflicts efficiently (Michod & Roze, 2001). As opposed to other multicellular lineages, where organisms had to struggle with these kinds of difficulties, early fungal hyphae were coenocytic (coenocyte is a type of cells which is a result of multiple nuclear division without cytokinesis), multinucleate tubular structures without any compartmentalization. Therefore, their evolution might have avoided group conflicts. There was either no need for fitness alignment between individual compartments and cells (Nagy et al., 2018), or these conflicts got resolved at the level of individual nuclei (Svedberg et al., 2018). Additionally, hyphae fill the available space and maximize foraging and nutrient assimilation efficiency by a fractal-like growth mode, forming mycelial networks (Fricker et al., 2017; Richards et al., 2017; Stajich et al., 2009). In these networks, hyphae rarely stick to each other and there is an efficient maximization of the distance between neighboring hyphae, which might help avoiding competition for nutrients. This growth mode of hyphae might have also facilitated the transition of fungal species to terrestrial life (Sekimoto et al., 2011) on contrary to other multicellular organisms, where selection for increased size served other purposes, for example helped avoiding predation, or capturing prey. Hyphal filaments aggregate and adhere to each other only in complex multicellular fruiting bodies of fungi (Kües et al., 2018; Pöggeler et al., 2018; Stajich et al., 2010), which, in terms of complexity levels resemble other multicellulars, metazoans and plants (Knoll, 2011; Nagy et al., 2018). In contrast to most multicellular lineages where cell-to-cell adhesion, intercellular cooperation and differentiation represent the main hurdles to the emergence of multicellularity (Abedin & King, 2010; King et al., 2003; Mendoza et al., 2013; Miller, 2012; Sebé-Pedrós et al., 2017), fungi represent a unique route to multicellularity. This uniqueness is can be due to their hyphal growth for which the regulation might have been the first step to the evolution of multicellularity, and adhesion – which was a key feature of animal multicellularity – for example, only becoming important in complex multicellular fruiting bodies or the attachment of hyphae to host surfaces.

1.4.2. Fossil records of early fungi and the emergence of hyphae

Understanding the early evolution of fungal species and estimate the time of their origins are challenging, due to the lack of their continuous fossil records, the presence of several

ambiguous early fossils (that are considered to be fungi), and the diversity of approaches used in the modern phylogenetic systematics and molecular clock analyses.

The most ancient Precambrian fossil evidences of fungi-like organisms come from a 2.4 billion-year-old basalt from Palaeoproterozoic Ongeluk Formation, South Africa (Bengtson et al., 2017), the 1 billion-year old Lakhanda Group, Siberia (Hermann & Podkovyrov, 2010), the 1,000-800 My old Grassy Bay Formation, Arctic Canada (Loron et al., 2019) and from the Mesoproterozoic Roper Group, Australia (Butterfield, 2005). Based on molecular clock analyses, these fossils are hundreds of millions of years older than the previously reported first occurrences of fungi (Berbee & Taylor, 2010). Accordingly, these fossils rewind the earliest fungal clade appearance back to an earlier time point than what was generally believed. However, the relationships of these Precambrian microfossils with real fungi remain uncertain. To date, the earliest and most convincing fossil evidences of fungi are 810-715 My old, and were found in the Mbuji-Mayi Supergroup, Democratic Republic of Congo (Bonneville et al., 2020). These mycelium-like fossils exhibit pseudoseptation and also branching, and represent the earliest, molecularly identified fossils of fungi.

The earliest fossil records of obligate biotrophic Glomeromycota-like hyphae resemble extant arbuscular mycorrhizal fungi (Glomeromycota). These remains originated from the Ordovician with an age of about 460 My (Redecker et al., 2000). Other relevant fungal fossils were found in the 407 My-old Rhynie chert from the early Devonian (Strullu-Derrien et al., 2018b), which is one of the most important deposit of fungal and plant fossil records. Fungal records found in Rhynie chert are highly diverse and abundant, representing Chytridiomycota (Taylor et al., 1992), Blastocladiomycota (Remy et al., 1994b), Glomeromycota (Remy et al., 1994a), Ascomycota remains (Taylor et al., 2005), and also provide evidences of early colonization of land (Dotzler et al., 2006, 2009; Kidston & Lang, 1921; Pirozynski & Malloch, 1975; Pressel et al., 2010; Remy et al., 1994a; Taylor et al., 2009a).

The earliest known fossil of zoosporic fungi, which develops hyphae and complex hyphal thallus is the *Retesporangicus lyonii* (Blastocladiomycota) from Devonian (Strullu-Derrien et al., 2018b), having extensively branched, complex hyphal thallus with intercalary or terminal sporangia. These newly evolved filamentous structures likely served as a saprotrophic adaptation to patchy resource availability (Strullu-Derrien et al., 2018b). Blastocladiomycota fossils show coenocytic hyphae and thalli with many centers at which reproductive structures are formed. The fossilized thalli have sympodial or

dichotomous branches and lack of septation or pseudoseptation. Globose to elongated zoosporangia are formed in small clusters, showing discharge papillae, and thick-walled resting spores represent the reproductive structures (Strullu-Derrien et al., 2018b, Remy et al., 1994b). Extant representatives of this group are the *Allomyces macrogynus* and *Catenaria anguillulae*.

Figure 5. Reconstruction of the 400 million-year-old Blastocladiomycota fossil fungus *Paleoblastocladia milleri*. Thallus at first level of dichotomy (48) and thallus with equal dichotomies of hyphal branches (49) (Figure is from Remy et al., 1994b)

Chytridiomycota is another early diverging fungal group where unicellular forms dominate, but hyphal forms are also represented (e.g. *Gonapodya prolifera*). The divergence of this group occurred around 750 million years ago (Mya), it constitutes less than 1% of extant fungi and possibly retains characters of the last common ancestor of fungi and animals, such as the unicellular body with cell wall, sporangium and uniflagellate zoospores (Keeling & Fast, 2002). Despite their unicellularity (e.g. *Batrachochytrium* and others with endoparasite lifestyle), some chytrid have developed small anucleate rhizoids that invade and colonize the substrate (Stajich et al., 2009). These chytrid rhizoids have been considered as possible precursors of the first fungal hyphae, and critical participants of the evolution of hyphal growth. Investigation of rhizoid morphogenesis in *Rhizoclosum globosum* seems to confirm these assumptions by showing that apical and fractal-like growth mode of rhizoids are very similar to that of fungal hyphae (Laundon et al., 2019).

The Zoopagomycota phylum consists of pathogens, parasites and saprobic fungi. Hyphal species are represented here, but there are exceptions. For example, the zoosporic fungus, *Olpidium* lacks either hyphae or rhizoids (James et al., 2006; Sekimoto et al.,

2011). With the exception of unicellular representatives, the coenocytic hyphae are typical, lacking regular crosswalls, or they are regularly septate without any perforation (e.g., *Basidiobolus* (Gull & Trinci, 1975), or septate with complex plugged septal pores (*Kickxellales*, *Asellariales*, *Dimargaritales* and *Harpellales* (Tanabe et al., 2004)). These structures, however, are distinct from those of Ascomycota and Basidiomycota septa.

Figure 6. The evolution of rhizoid morphogenesis and hyphal development (Figure is from Laundon et al., 2019).

The evolutionary relationships of Mucoromycotina, Entomophthoromycotina, Zoopagomycotina, Kickxellomycotina (Zygomycota) and Glomeromycota is not completely resolved yet, but they share similarities in their hyphal growth. They all grow primarily as coenocytic hyphae (or as yeasts in some cases) and septum formation is rare. *Palaeoendogone gwynne-vaughaniae* is a known fossil of Mucoromycotina, with aseptate, branched hyphae (Strullu-Derrien et al., 2014). In contrast to most zygomycetes, Kickxellomycotina fungi develop regular septa with central pores which become plugged with age. The development of regular septation, coupled with a shift in the method of spore formation (conidia formation by hyphal septation) in these fungi might have been

a key evolutionary event, because all Ascomycota and Basidiomycota fungi share these features (Stajich et al., 2009).

The Dikarya subkingdom contains the two biggest hypha-forming phyla of fungi, the Ascomycota and Basidiomycota. In both groups, the mitosis is followed by regular septum formation and they also possess septal pores (lenticular cavities). These central pores of septa regulate the passage of cytoplasmic components between the neighboring hyphal cells. In Basidiomycota, these pores involve membranes, while in Ascomycota, Woronin bodies regulate this cytoplasmic flow between compartments (Jedd, 2011; Stajich et al., 2009).

Figure 7. Fungal tree of life based on published multi-gene and genome-scale phylogenies (Figure is from Spatafora et al., 2017)

Although hyphal forms dominate in both phyla, there are lineages where the secondarily simplified yeast form is common (Saccharomycotina, Taphrinomycotina in Ascomycota and Ustilaginomycotina, Tremellomycetes and Pucciniomycotina in

Basidiomycota). However, several unicellular forms are capable of forming filamentous structures (either pseudohyphae or true hyphae) in response to environmental signals. In pseudohyphae the chains of the budding cells remain connected, and despite the similarity to true filaments, the septa between pseudohyphal compartments have no pore, therefore it lacks cytoplasmic connections. These different morphological terms like “yeast”, “pseudohyphae” and “true hyphae” are practical in fungal research and are frequently used, however the distinction between yeast forms and pseudohyphae and between pseudohyphae and true hyphae is difficult, the boundaries of these states are not clear, and may be better to consider them as a continuum (Merson-Davies & Odds, 1989).

Based on the well-preserved fossil records, filamentous fungi are thought to be absent until the middle of the Palaeozoic era lasting from 540-250 Mya. However, recent discoveries completely changed our knowledge about the origin of fungi and hyphal growth, and further investigations are needed to answer our emerging questions about when and how multicellular fungi evolved. Although the exact time of the emergence of filamentous fungi is yet unresolved, the emergence of filamentous hyphal growth was considered to be a major step in fungal evolution, providing an incredibly large evolutionary advantage in the continuously changing environment. Thanks to the evolution of hyphal growth, fungi acquired new capabilities. They were able to adopt invasive growth to infect larger organisms during pathogenesis, and also increase the surface in saprotrophic lifestyle to improve the search for fresh food in either nutrient-rich or nutrient-poor, living or dead plant or animal tissues (Berbee et al., 2017). In addition, they were able to expand enzyme repertoires for metabolite production and secretion, and express membrane transporters. These innovations are hypothesized to be the direct consequences of terrestrial lifestyle (Naranjo-Ortiz & Gabaldón, 2019), and they are also related to the emergence of osmotrophy (Richards & Talbot, 2018). Parallel to polarized growth, the evolution of regular septation at the Zygomycota/Dikarya split also opened the door for the evolution of different hyphal segments with different functions and tissues in complex multicellular fungi (Stajich et al., 2009).

1.4.3. Ecological and industrial importance of filamentous fungi and hyphal growth

1.4.3.1. Terrestrialization

Filamentous fungi have conquered different ecological niches during their evolution and probably played a key role in the early terrestrialization of plants by their symbiotic/mutualistic interactions (Kidston & Lang, 1921; Pirozynski & Malloch, 1975; Pressel et al., 2010; Selosse & Tacon, 1998; Selosse et al., 2004; Taylor et al., 2009a; Wang et al., 2010). The first land plants colonized Earth in the Ordovician, and the associations between land plants and fungi are thought to date from this time (Dotzler et al., 2006, 2009; Kenrick et al., 2012; Remy et al., 1994a; Remy et al., 1994b; Taylor et al., 2014; Taylor et al., 2009b). Fossil evidences of these interactions are known from the previously mentioned Rhynie chert from the early Devonian, including fungal-plant associations of early diverging fungal lineages Chytridiomycota, Blastocladiomycota, Mucoromycotina and the Glomeromycotina. Globular forms with rhizoids were identified in the Rhynie chert as chytrid fungi, but none of them were directly associated with plants (Strullu-Derrien et al., 2018a). Blastocladiomycota fossils, on the other hand showed fungal-plant association, where the fungus colonized the degraded plant material of *Aglaophyton majus* possibly via having a saprotrophic lifestyle (Remy et al., 1994b), or colonized the rootlets of *Asteroxylon mackiei* as a parasite (Strullu-Derrien et al., 2015). Beside these examples, many zoosporic fungi are found in Carboniferous cherts as well, indicating their possible roles in early forest ecosystems (Strullu-Derrien, 2018).

Glomeromycota is one of the most important fungal groups, because they produce arbuscular mycorrhizal interactions with more than 90% of plant species, contributing to nutrient acquisition, growth and stress tolerance as well (Begum et al., 2019). The first fossil evidence of these fungal-plant associations is a glomalean fungus from the Ordovician with the age of 460 My, suggesting that Glomeromycota fungi were present before the first vascular plants colonized land (Redecker et al., 2000). Another example is a 400 My-old vesicular arbuscular mycorrhiza (AM). AM was also identified in the Rhynie chert plant *Aglaophyton majus*, with intracellular arbuscules and intercellular vesicles in its prostrate axes (Remy et al., 1994a). Most of these early mycorrhizal associations are similar to those observed in extant nonvascular plants and different from root-associations, and the first evidences of root-colonization come later, from Carboniferous cherts, well-documented in *Cordaitea* (Strullu-Derrien et al., 2009).

Nevertheless, arbuscular mycorrhizae are only one of the many fungus-plant associations. Two other types of endomycorrhizae also exist, involving orchid and ericoid mycorrhizae. In both cases the root cells are colonized by hyphal coils and the fungal sheath is absent. These interactions between fungi and plants can range from mutualistic

to parasitic relationships and facilitate mostly carbon-, nitrogen- and phosphorus-transport between partners. Ectomycorrhizae (ECM) are another type of mycorrhizae – dating back to 48.7 Mya – where hyphae form sheath and Hartig-net between cells and extend intercellularly through the cortex to the endodermis. In these interactions, the fungal symbiont such as Ascomycota, Basidiomycota and few Mucoromycotina (*Endogone* genus) colonizes mostly shrub and tree roots in temperate, boreal and Mediterranean forests (Strullu-Derrien et al., 2018a). These fungal interactions facilitate the nutritional adaptation and global diversity of around 80% of living plant species and 95% of Angiosperm plants (arbuscular mycorrhizae: 74%, orchid mycorrhizae: 9%, ectomycorrhizae: 2%, ericoid mycorrhizae 1%) (Brundrett, 2009; Martin et al., 2016).

1.4.3.2. Decomposition and recycling

Thanks to their hallmark features (enzyme production and secretion), filamentous fungi have a key role in the decomposition and recycling of litter, organic soil material and plant cell wall material. The degradation of wood, straw and other organic components found in biomass is mainly done by saprotrophs and wood decayer (white, brown and soft rot) fungal species (Evans et al., 2001; Evans & Hedger, 2009). These processes require a production and secretion of a large and comprehensive array of enzymes responsible for degradation, including lignin peroxidases, laccases, different carbohydrate active enzymes (CAZymes), pectinases, cellulases and hemicellulases, just to name a few.

1.4.3.3. Biotechnology

During the last decades, biotechnology discovered the usefulness of filamentous fungi. These organisms are highly productive cell factories with extended secretion capacity. The secreted primary and secondarily metabolites consists of various proteins, enzymes, antibiotics and organic acids. Thanks to their hyphal growth, filamentous fungi can effectively colonize substrates, and due to the large surface to volume ratio, the uptake of supplements is quite efficient (Wösten, 2019). With the improvement of these beneficial features using modern molecular biology techniques, it was reasonable to build a profit-oriented industry on filamentous fungi and their metabolite production.

Nowadays several products for drug, food, paper and textile processing is produced by filamentous fungi which serve as cell factories (*Trichoderma*, *Aspergillus*, *Penicillium* sp.) (Peberdy, 1994; Wösten, 2019). Also, recent discoveries on renewable, mycelium-based materials open the door for an environmentally friendly, low-cost and minimum-energy production of new materials, and the opportunity to use them as an alternative to plastics and other petroleum-based materials (Haneef et al., 2017).

1.4.3.4. Pathogenesis

Besides the ecological importance of filamentous fungi, and the huge benefit of industrially applicable species, some fungi considered as a severe threat to public health and agriculture (Brown et al., 2012). Hyphal growth can serve as a key virulence factor in fungal pathogenesis, like in superficial mycoses, invasive mucoromycoses, plant and insect infections (Gauthier, 2015). Dimorphism is the ability of fungi to switch between unicellular yeast and multicellular filamentous form in response to environmental cues such as changes in temperature, pH, or the presence of pheromones. This morphogenetic shift in the mode of growth is essential for dimorphic fungal pathogens to adopt to the new conditions, spread and colonize the host and invade surrounding tissues (Gauthier, 2017; Nadal et al., 2008). There are several examples of dimorphic fungi where hyphal growth is considered to be a virulence factor.

Ustilago maydis, a basidiomycete phytopathogen is responsible for corn smut disease. This fungus is able to switch between saprobic, haploid, yeast stage and pathogenic dikaryotic filamentous form in response to nuclear condition and plant signals. The filamentous form develops exclusively *in planta* after the mating of compatible sporidia. The proliferation and the resulting protein secretion suppress host defense and induce tumors in maize plants (Brefort et al., 2009). *Ceratocystis (Ophiostoma) ulmi*, the causative agent of Dutch elm disease is another example for dimorphic plant pathogens. Here the yeast-hypha transition is also regulated by environmental signals. Yeast cells are involved in dissemination and passively transported in plant vessels, whereas the filamentous forms penetrate from one vessel to another (Brasier, 1991). Other notable examples of the more than 100 dimorphic phytopathogen species are *Taphrina deformans* (peach and almond leaf curl), *Mycosphaerella graminicola* (pathogen of wheat), and *Holleya sinecauda* (pathogen of

mustard seeds). In each of the abovementioned cases, the filamentous form is responsible for the plant pathogenesis (Nadal et al., 2008).

Candidiasis is one of the most common fungal infection in humans, leading to both life-threatening invasive and non-life-threatening mucocutaneous diseases. It causes mostly vaginal, oral or epithelial mucosal infection in susceptible humans (Kim & Sudbery, 2011). The opportunistic fungal pathogen, *Candida albicans* is one of the prominent examples of the disease-causing *Candida* species, which have the capacity for morphogenetic switch in response to environmental fluctuations in human bodies. This switch is a crucial virulence factor of *Candida* species (Sudbery et al., 2004; Sudbery, 2011). The mycelial form is important for the invasion into hosts and the yeast forms help the dissemination of the fungus and thereby enables the spread of the disease. Hypha formation is also a key feature of candida biofilms where adherent cells stick to each other and form a stable and irreversibly attached layer on different surfaces, such as living tissues or inert materials (Finkel & Mitchell, 2011; Ramage et al., 2005). Due to their high resistance to antifungal treatments, and their ability to continuously release infective cells, these biofilms cause severe clinical problems. Contaminated catheters, prostheses are also suitable substrates for biofilm formation.

Malassezia globosa, a dermatotrophic fungus can cause another type of disease by using a similar “virulence toolkit”. The yeast form is a facultative intracellular parasite of keratinocytes and suppresses inflammatory response. Short hyphae on the other hand penetrate keratinized skin cells to invade deeper. The hyphae of these fungi helps rerooting the infection in nutrient-rich, deep and cornified layers to replace the older fungal colonies for fresh ones (Brand, 2012). Other keratinolytic dermatophytes, *Trichophyton mentagrophytes*, *Arthroderma benhamiae* use similar mechanisms for pathogenesis.

The abovementioned examples are only a few of the hundreds of filamentous fungal pathogens. The investigation of filamentous fungi and hyphal growth has an enormous impact on public health and agriculture, and the new discoveries related to this topic can play an important role in prevention and treatment of infectious diseases caused by filamentous fungi.

1.5. Molecular and cellular basis of hyphal morphogenesis

Hyphal growth involves several cellular pathways and entailed the evolution of different novel traits, including notable examples of the establishment and maintenance of cell polarity generating continuous polarized tip-extension, the coordinated transport of cell wall material to the sites of active growth, and the establishment of branching and septation patterns.

The filamentous, continuously growing shape of fungal hyphae is achieved by the localized expansion of the hyphal tip. This expansion is predominantly driven by a tip-localized Ca^{2+} gradient and the turgor pressure within the cell (Lew, 2011). Cell membrane and cell wall extension generate elevated intracellular Ca^{2+} concentration which mediates vesicle fusion required for the continuous cell expansion. This Ca^{2+} -mediated vesicle fusion is probably controlled by SNARE family proteins (Gupta et al., 2003; Gupta & Heath, 2000) at the hyphal tip. Osmolyte synthesis, another mechanism required for turgor regulation, on the other hand, is fine-tuned by the osmotic mitogen-activated protein kinase (MAPK) pathway, which appears to be ubiquitous in fungi (Brewster et al., 1993; Kranz et al., 2006).

A widely held view is that the hyphal cell wall at the apex has to be plastic in contrast to other parts of the hyphae (Wessels, 1986). During cell growth, cell wall-lytic enzymes have to loosen the wall at the apex to allow the insertion of newly synthesized cell wall material. As an alternative, the steady-state model of hyphal growth (Wessels, 1988) assumes that this extension depends on the continuous extrusion of visco-elastic wall material which is subsequently hardened by cross-linking processes in the cell wall. The rate of extrusion of polymers into the cell wall and the rate of rigidification are supposed to be independent (Wessels, 1988). The rapid rate of hyphal extension requires a large amount of cell wall components deposited at the extension site. Plastic cell wall material (chitin, β -1,3-glucan, β -1,4-glucan, β -1,6-glucan, α -1,3-glucan) has to be synthesized and delivered continuously to the apex to elongate the hypha. After deposition, cross-linking mechanisms rigidify the proto cell wall material, and only the hyphal apex is plastic enough to grow continuously. This process is coordinated by various cell wall-synthesizing and modifying enzymes and proteins: glucan and chitin synthases (FKS1 in *Saccharomyces cerevisiae*, AGS1 in *Schizosaccharomyces pombe*, CHSB, CHSD, CHSE in *Aspergillus nidulans* and CHS1 in *Neurospora crassa*), glycosyl

hydrolases and GPI proteins (GAS/GEL/PHR in *Ustilago maydis*, CRH, BGL2 in *Saccharomyces cerevisiae*, ECM33 in *Candida albicans*) (Klis et al., 2007).

The establishment and maintenance of the polarity axis are the two key events underlying the spatial regulation of hyphal morphogenesis. Specification of the growth site relies on positional cell end markers (TEA4 in *Ustilago maydis*, TEAC in *Aspergillus nidulans*) and protein complexes which are connected to downstream morphogenetic signaling pathways (Fischer et al., 2008; Harris & Momany, 2004; Momany, 2002; Rittenour et al., 2009; Takeshita et al., 2008; Virag et al., 2007; Wendland, 2001).

After the axis is specified by polarity markers, the subsequent maintenance of this growth axis requires the continuous recruitment of morphogenetic machinery components to the growing site, producing localized cell surface expansion and cell wall deposition. One of the core signal transduction pathways connected to these events is the RAS/RHO GTPase pathway well described in *Saccharomyces cerevisiae*, but having conserved components in filamentous fungi as well. This pathway is responsible for polarity establishment and maintenance by the formation of a stable polarity axis that specifies the site of germ tube emergence. This positional information is generated by the recruitment of cell polarity proteins (AXL2 and BEM3 in *Saccharomyces cerevisiae*, SWOC, TEAR in *Aspergillus nidulans*) at the site of polarized growth and then is transduced via regulatory RHO GTPases (RHO1, RHO4, RACA in *Aspergillus fumigatus*, MODA in *Aspergillus nidulans*, CDC42 in *Saccharomyces cerevisiae* and *Candida albicans*) toward the morphogenetic machinery, which is the main organizer of tipward secretion, cell wall expansion, and polarized growth in fungi (Wendland, 2001). The RAC-CDC42-CDC24 GTPase module is strongly coupled with the cytoskeletal network, therefore the regulation and remodeling of the actin cytoskeleton has a large impact on polarized growth. The RAC-CDC42-CDC24 GTPase module indirectly regulates the actin cytoskeleton via other polarity-establishing and maintaining factors: the polarisome and the ARP2/3-WASP multiprotein complex. Both of these complexes have a role in actin polarization. The polarisome complex have been identified in yeast, organizes cytoskeletal components to the expansion site and regulates cytoskeleton-mediated transport of vesicles (Lin et al., 2014; Riquelme et al., 2018) by formin-dependent (SEPA and MESA in *Aspergillus nidulans*, SPA2, BUD6, and BNI1 in *Neurospora crassa*) microfilament nucleation and actin polarization. SPA2 polarisome protein localizes to hyphal tip in *Ashbya gossypii*, *Candida albicans* and *Aspergillus nidulans*, and many other components of the polarisome (for example the formin SEPA)

forms a dynamic structure with the microfilament-organizing center called Spitzenkörper (see its detailed description later) (Harris et al., 2005). Polarisome complex is also involved in hypha orientation along with calcium signaling, TEA cell-end marker proteins, specific kinesins and sterol-rich apical microdomains. Mutations in the contributing genes result in normal-shaped growing hyphae with abnormal meandering trajectories or attenuated tropic responses. The strong cooperation between different hyphal complexes show that these distinct regulatory mechanisms, the regulation of tip orientation and tip extension operate in parallel during polarized growth (Brand & Gow, 2009).

The ARP2/3-WASP complex in turn controls the formation of branched actin filaments and has a role in building the cortical actin patches at the apex which also have a crucial role in endocytosis. Key components of this complex in yeast are the WASP homologue LAS17/BEE1, the class I myosin MYO3 and the actin-related proteins ARP2, ARP3, all of them are conserved in filamentous fungi. In yeasts, actin patches mainly contribute to endocytosis, which retrieve and recycle the positional markers and morphogenetic machinery components, promoting polarized bud growth (Harris & Momany, 2004). In filamentous fungi, the role of actin patches is likely similar, the actin patches are formed as peripheral structures at the subapical regions, and possibly have role in the coupling of apical exocytosis and the subapical endocytosis (Peñalva, 2010; Steinberg, 2014), and therefore in the endocytic recycling of the cell material (Araujo-Bazán et al., 2008; Shaw et al., 2011). Fungal exocytosis is a critical part of the hyphal elongation. All substrates required for apical extension are delivered to the tip by exocytosis, but it also has to be balanced by endocytosis, which was thought to be absent in fungi for many years (Read & Kalkman, 2003). However recent studies revealed the critical role of endocytic recycling and early endosome (EE) motility in hyphal morphogenesis (Peñalva et al., 2012; Steinberg, 2014; Steinberg et al., 2017).

According to these discoveries, the bi-directional, microtubule-based, long-distance transport of early endosomes along with the help of motor protein kinesins and dynein is an indispensable element of the morphogenetic machinery. This transport is required not only for polarized growth, but also for septin filament formation and protein translation. For example, the mRNA of septin CDC3 and CDC12 are actively-translated on the moving early endosomes, and it is likely that the assembly of septin complexes also occurs on endosomes. This EE-associated translation supports septin filament

formation which is a key process in filamentous growth (Baumann et al., 2014; Steinberg, 2014).

During expansion, the transport of growth supplies (membrane and cell wall components, enzymes, signaling molecules etc.) is required. Cell wall materials are transported by secretory vesicles to the growing tip along cytoskeletal structures (Steinberg, 2007a, 2007b). The key contributor, which organizes the movement of these secretory vesicles is the Spitzenkörper (Arkowitz & Bassilana, 2011; Riquelme et al., 2018; Riquelme & Sánchez-León, 2014; Steinberg, 2007b; Steinberg et al., 2017; Takeshita, 2016; Virag & Harris, 2006), composed of a collection of macro- and micro-vesicles. It is assumed to serve as a vesicular distributor center between the microtubular transport system and the actin cytoskeleton near the hyphal apex, and therefore it is responsible for the growth directionality of hyphae. The vesicles - distributed by the Spitzenkörper – transport several biomolecules that are essential for hyphal extension, such as structural landmark proteins that facilitate membrane docking and fusion, as well as cell wall biosynthetic and remodeling enzymes, lipids, polysaccharides and Ca^{2+} . The actin cytoskeleton has a crucial role in taking vesicles delivered to the apex via myosin-based, long-range vesicular transport and guiding them to polarization and secretion sites, serving as a bridge between the Spitzenkörper and the extension site of the hyphal apex. Precise coordination of this vesicle delivery to the expansion site is essential for polarized growth. The key regulators of this process are the I.) coatomer complex that mediates vesicle formation (Orci et al., 1993), II.) the exocyst complex (SEC5, SEC6, SEC15, EXO70 etc. in *Aspergillus nidulans*, EXO-70, and EXO-84 etc. in *Saccharomyces cerevisiae*), which is a conserved multiprotein complex having a role in vesicle targeting and vesicle distribution from the Spitzenkörper to exocytic routes (Lin et al., 2014; Riquelme et al., 2018; Steinberg et al., 2017; Takeshita, 2016), and finally, III.) the SNARE complex (SYN1 and SYN2 in *Neurospora crassa*, (Gupta et al., 2003)), which facilitates vesicle fusion with specific membrane types. The Spitzenkörper is present across all Dikarya and in certain early-diverging fungi, such as *Allomyces*, *Blastocladiella* in Blastocladiomycota (James et al., 2014; Mcdaniel & Roberson, 1998; Srinivasan et al., 1996; Vargas et al., 1993). The Zygomycota lineage lacks the SPK in general and has a less-organized vesicle aggregation apical vesicle crescent (AVC) (Fisher & Roberson, 2016). Notable exceptions, when the SPK is present are the *Basidiobolus* (Roberson et al., 2011) and *Conidiobolus* species (Fisher et al., 2018). Evolutionary analysis of the SPK shows that the Spitzenkörper was present in the common ancestor of fungi and

probably lost or modified in Zygomycota. An alternative hypothesis suggests that the SPK has an independent origin in different fungal lineages (Naranjo-Ortiz & Gabaldón, 2019).

Septation and branching patterns are also characteristic morphological features of filamentous fungi. Cells in hyphae divide by the process of septation, during which a cross-wall is synthesized between the two neighboring cells that remain to be connected by septal pores in Ascomycota and Basidiomycota. Mitotic signals and cortical markers are the inducers of septation, and the morphogenetic machinery behind this process is composed of the contractile actin ring (CAR), the septation initiation network (SIN), septins (ASPA-E in *Aspergillus nidulans*, CDC10 in *Candida albicans*), and formins (BNI1 in *Neurospora crassa*, FOR3 in *Schizosaccharomyces pombe*) (Steinberg et al., 2017). Septation and branching are two morphogenetically connected events. In septum-forming fungi, the branching event occurs only after septation and starts with the generation of a new polarity axis, followed by the recruitment of the morphogenetic machinery to induce branch emergence. In *Neurospora crassa*, the characteristic POD proteins (POD4, POD5, POD8) are required for branch formation, but interestingly they have no obvious role in the morphogenesis of primary hyphae (Harris, 2008).

Not only spatial but also temporal coordination is needed to maintain the nuclear content of uninucleate hyphae. The precise coordination of morphogenesis with nuclear division is controlled mainly by cyclin-dependent kinases (CDKs). For example, in *Saccharomyces cerevisiae* the CDC28 kinase coordinates the polarity and cell division during the cell cycle (Edgington et al., 1999). CDKs control the timing of polarity establishment and septum formation by the regulation of septins and polarisome components (Zou et al., 2009). On the other hand, in multinucleate hyphae, this coordination is not that obvious. Nuclear division mutants of *Aspergillus nidulans* are capable of forming polarized hyphae, despite the fact that the cell division machinery suffers from defects (Morris, 1975). But other evidences show the opposite, *nim* (never in mitosis) mutants of *Aspergillus nidulans* fail to establish polarity (Bergen et al., 1984; Harris et al., 1999; Morris, 1975).

2. Objectives

In the last decade we have entered the “genomic era”. The improvement of genome sequencing technologies enabled us to sequence hundreds of genomes and produce enormous and diverse genomic data. The aim of my work was to answer the arising questions about the evolution of hyphal multicellularity and hyphal morphogenesis using cutting edge bioinformatics approaches, especially comparative genomic methods.

Our knowledge on the evolutionary origin of hyphal multicellularity is far from complete. Nevertheless, the investigation of extant fungal genomes enables us to perform evolutionary genomic analyses and examine the evolution of hyphal multicellularity. With the help of the newest computational techniques, I reconstructed historical patterns of known hypha morphogenesis-related gene families. I also performed systematic investigation of fungal genomes with the aim to seek for gene families whose evolution correlates with the evolution of hyphal multicellularity. Using an extensive taxon sampling I investigated the genomic and developmental commonalities among multicellular lineages and answer how hyphal multicellularity differs from other types of multicellularity.

During my doctoral thesis, my main goal was to address the following questions:

1. What are the evolutionary origins of hyphal multicellularity?
2. Is there any major genetic innovation behind the transition from unicellular to multicellular fungi?
3. Is there any ancient genetic toolkit that plays a role in hyphal multicellularity?
4. What are the functional and molecular predispositions to hyphal multicellularity?
5. How does hyphal multicellularity differ from other types of multicellularity in other lineages?

3. Methods

3.1. Taxon sampling

Taxon sampling was designed in a way to cover all major clades of fungi (following the phylogenetic classification of (Spatafora et al., 2016), irrespective of their cellularity status (unicellular or hyphal multicellular), to avoid any bias in the dataset towards hyphal species. Accordingly, we selected species from all three subphyla of both the Ascomycota (Taphrinomycotina, Saccharomycotina and Pezizomycotina) and the Basidiomycota (Agaricomycotina, Pucciniomycotina and Ustilaginomycotina), as well as a representative set of Mucoromycota, Zoopagomycota, Chytridiomycota, Blastocladiomycota and Cryptomycota. We omitted Microsporidia from the dataset due to the rapid rate of evolution of this group (Slamovits et al., 2004). As outgroups, multicellular animals, their unicellular relatives and other non-fungal eukaryotes were also included in the analyses. Using these outgroups enabled us to compare multicellularity-related gene families across kingdoms.

3.2. Organismal phylogeny

We assembled a dataset containing whole proteomes of the investigated 71 species and performed all-vs-all blast using mpiBLAST 1.6.0 (Darling et al., 2003). Protein sequences were clustered into gene families based on sequence similarity by following the MCL clustering protocol (van Dongen, 2000) used by (Ohm et al., 2012) with an inflation parameter of 2.0. Clusters with at least 50% taxon-occupancy were chosen and protein sequences per clusters were aligned by PRANK 140603 with default settings (Löytynoja, 2014), while trimAl 1.4.rev15 (Capella-Gutierrez et al., 2009) was used for alignment trimming to remove poorly aligned regions from the multiple sequence alignments using the parameter `-gt 0.2`. Approximately-maximum-likelihood gene trees were inferred by FastTree 2.1.10 (Price et al., 2009) using the LG + CAT model (`-lg -cat20`) of protein evolution, and the option `-gamma` to compute Gamma20-based likelihoods. Single-copy genes are generally used for inferring phylogenetic relationships between taxa. In order to use only the single-copy gene trees for our phylogenetic reconstruction, we excluded gene trees that contained deep paralogous genes (see

(Prasanna et al., 2019) for details). Filtering deep paralogs was performed by a custom-made Perl script that analyzes gene duplication patterns in inferred gene trees to identify single-copy genes. The gene trees of not strictly single-copy genes were checked if the duplications are deep in the tree, or species specific. In those cases where the gene duplications were species specific, the gene was marked as suitable for phylogenetic inference and the gene that was closest to the root (in terms of patristic distance (i.e., divergence between lineages)) was chosen for inclusion in the phylogenetic analyses. Alignments of single-copy gene clusters were concatenated into a supermatrix using a custom-made Perl script and a Maximum Likelihood (ML) species tree was inferred for the 71 species using RAxML 8.2.4 (Stamatakis, 2014) under the PROTGAMMAWAG model. The model was partitioned by gene and bootstrapping was performed on the dataset in 100 replicates.

3.3. Ancestral character state reconstruction

The 71 species in the phylogeny were coded for their ability to form multicellular hyphae. Two categories were made, one for hyphal and one for non-hyphal species. Species that could not be unambiguously assigned to hyphal or non-hyphal state (e.g. *Catenaria anguillulae*) and those with the ability to grow either as hyphae or unicells (most yeasts) were coded as uncertain. Bayesian MCMC reconstruction of the ancestral character states was performed under the threshold model (Felsenstein et al., 2012) using Bayesian MCMC with the “ancThresh” function in phytools v0.6-60 (Revell, 2012, 2014) in R (Ihaka & Gentleman, 1996). The number of generations for MCMC was set to 1,000,000, and the method “mcmc” was used with the Brownian motion as the model for the evolution of the liability. Burn-in parameter was set to default and the convergence was checked by inspecting likelihood values through time.

3.4. Identification of hyphal multicellularity-related genes

Genes related to hyphal morphogenesis and multicellularity were collected based on literature search. Genes were regarded as “hyphal morphogenesis/multicellularity-related” if knock-out or transcriptional studies confirmed the connection, or strong hypotheses supported the link between the gene and the morphogenetic change. Collected

genes were classified into 10 functional categories based on subjective categorization, as follows: “actin cytoskeleton regulation”, “adhesion”, “cell cycle”, “cell wall biogenesis and remodeling”, “microtubular transport”, “polarity establishment and maintenance”, “septation”, “signaling”, “transcriptional regulation” and “vesicle transport”.

3.5. Analysis of gene family evolution

To investigate the evolutionary origin of hyphal multicellularity-related gene families and to recover their gene duplication and loss histories, we analyzed protein clusters (gene families) that contained such hypha morphogenesis-related genes based on the above described MCL clustering of the proteomes of the 71 analyzed species. The resulting clusters were considered as hyphal multicellularity-related gene families and the belonging proteins were aligned by PRANK 140603 (Löytynoja, 2014) with default parameters, and ambiguously aligned regions were removed using trimAl 1.4.rev15 (Capella-Gutierrez et al., 2009) with the argument `-gt 0.2`. MAFFT v7.222 (Katoh, 2002) (option `--auto`) was used as an alternative alignment tool for clusters that could not be aligned by PRANK (80 out of 34032 clusters) due to computational limitations. Maximum Likelihood inference of gene trees and calculation of Shimodaira-Hasegawa-like (SH-like) branch support values were carried out in RAxML 8.2.4 (Stamatakis, 2014) under the PROTGAMMAWAG model of protein evolution. The calculated SH-like branch support values were used in gene tree-species tree reconciliation in Notung-2.9 (Chen et al., 2000). An edge-weight threshold of 0.9 was used as SH-like support values are usually less conservative than Maximum Likelihood bootstrap values (where 70% is usually taken as indication of strong support). Gene tree-species tree reconciliation was performed on the ML gene trees and the ML species tree for the 71 species as input. We then reconstructed the gene duplication and loss dynamics of each morphogenesis-related gene families along the species tree using ortholog coding method and Dollo parsimony mapping from the COMPARE pipeline (Nagy et al., 2014; Nagy et al., 2016) (scripts are available at <https://github.com/laszlognagy/COMPARE>). The numbers of gains and losses for each gene family and for each branch of the species tree were recorded and mapped on the species tree. Ancestral gene copy numbers were calculated for every internal node, summing the mapped gene duplications and losses across the species tree.

Mappings were generated for each of the investigated gene families, for functional groups, as well as for all gene families across the 71 genomes.

3.5.1. Enrichment analysis of gene duplication events

To test if the hyphal morphogenesis-related genes experienced an episode of increased duplication rate in those ancestral nodes where hyphal growth putatively originated (BCZ nodes, that represent the split of Blastocladiomycota, Chytridiomycota and Zoopagomycota lineages), we performed gene duplication enrichment analysis for each of the 362 gene families and for the 10 functional categories. To test if a gene family/cluster or a functional group shows significantly more or less duplications than expected by chance in BCZ nodes, we performed two-tailed Fisher's exact tests ($p < 0.05$). We compared the number of duplications mapped to BCZ nodes for a given gene family/functional group to the genome-wide number of duplications in BCZ nodes, using total number of duplications across the tree as a reference.

Figure 8. Pipeline of the analysis of gene family evolution of hyphal multicellularity-related genes

3.5.2. Analyses of key multicellularity-related gene families

The same strategy was used to reconstruct the evolutionary history of histidine kinase, serine-threonine kinase, G-protein-coupled receptor (GPCR) and adhesion-related gene families. Protein clusters containing kinase genes, both serine-threonine kinases and histidine kinases were collected from the protein clustering of the analyzed 71 species, based on characteristic InterPro domains. Identification of serine-threonine kinases and histidine kinases followed Hérivaux's and Park's identification (Hérivaux et al., 2016; Park et al., 2011). Classification of histidine kinases followed Defosse's classification (Defosse et al., 2015). Families of adhesive proteins were identified based on experimentally characterized genes collected from the literature. We identified 45 genes, which mostly grouped into flocculins, lectins, hydrophobins and glycosylphosphatidylinositol (GPI)-modified cell wall adhesins. We identified GPCR genes based on InterPro (IPR) domains that were annotated with the gene ontology term 'receptor activity' (but not 'receptor binding' or other terms indicative of indirect relationships to receptor function), resulting in 27 IPR terms (IPR000161, IPR000276, IPR000337, IPR000363, IPR000366, IPR000481, IPR000832, IPR000848, IPR001103, IPR001105, IPR001499, IPR001546, IPR001946, IPR002011, IPR002185, IPR002280, IPR002455, IPR002456, IPR003110, IPR003292, IPR003980, IPR003982, IPR005386, IPR006211, IPR017978, IPR017979, IPR017981). Gene duplication and loss mappings were generated for histidine kinase, serine-threonine kinase, adhesion-related protein as well as for GPCR coding gene families, using the COMPARE pipeline.

3.5.3. Analyses of phagocytosis-related gene families

We collected information on phagocytosis-related genes from recent reviews both on the phagocytotic mechanisms of *Dictyostelium discoideum* (Bozzaro et al., 2008; Cardelli, 2001; Rivero, 2008) and other eukaryotes (Mao & Finnemann, 2015). The corresponding genes of these species were identified in our 71-genome dataset and the protein clusters that contained orthologs of the identified genes were analyzed. Mapping of gene duplications and losses along the species tree was performed using the COMPARE pipeline.

3.6. Genome-wide screen for novel hypha morphogenesis-related gene families

To identify gene families with increased rates of gene duplication coinciding with the origin of hyphal multicellularity, we set up a pipeline that tests for higher than expected rate of duplication in nodes of the species tree to which the origin of hyphae could be located (BCZ nodes). For each gene family, gene duplication rates in BCZ nodes were compared to duplication rates of the same family in other parts of the species tree (nodes before and after BCZ nodes). Gene duplication rates were computed by dividing the number of reconstructed duplications for a given branch by the length of that branch using a custom Perl script. Terminal duplications and duplications mapped to metazoan ancestors were excluded from the analysis. The resulting node $\times$ duplication rate matrix was analyzed by a two-factor permutation ANOVA (Nagy et al., 2016) with degrees of freedom DFT = 2, in R, with $p < 0.05$ considered as significant. Further requirement was that the detected clusters should be conserved (≥ 1 copy) in at least 70 % of filamentous fungi.

3.7. Analyses of gene losses in yeast-like fungi

We analyzed gene losses in five yeast-like lineages (Saccharomycotina, Taphrinomycotina, Ustilaginomycotina, Tremellomycotina and Pucciniomycotina) by comparing the number of gene losses genome-wide, to the numbers of gene losses in hyphal morphogenesis-related genes (in “actin cytoskeleton regulation”, “polarity establishment and maintenance”, “cell wall biogenesis/remodeling”, “septation and septal plugging”, “signal transduction”, “transcriptional regulation”, “vesicle transport”, “microtubule-based transport” and “cell cycle regulation” categories) relative to ancestral copy numbers. P-values were calculated by Fisher’s exact test, with $p < 0.05$ considered as significant. The percentage of genes retained in yeast genomes was calculated for every functional category by comparing ancient gene-copy number prior to the emergence of yeast-like lineages to the average gene copy-number of terminals.

4. Results

4.1. Hyphae evolved in early fungal ancestors

To understand the evolutionary origin of multicellular hyphal growth, a species phylogeny was constructed by maximum likelihood analysis, using publicly available genomes. This phylogeny represents 71 species, including 4 plesiomorphically unicellular, 39 hyphal (one of which is ambiguous: *Catenaria anguillulae*) 15 secondarily simplified unicellular (yeast-like) fungi, as well as 13 non-fungal relatives containing slime molds, amoebas and animal species (see species names, accession numbers and information about their complexity levels in Appendix 1.). The inferred phylogenetic tree for the 71 species was based on a supermatrix of 75,224 sites from 455 single-copy ortholog genes (Figure 9.). Our species phylogeny is strongly supported and recapitulates recent genome-based phylogenies of fungi (Figure 7.) (Ahrendt et al., 2018; Chang et al., 2015; Spatafora et al., 2016, 2017). We observed that Rozellomycota, Blastocladiomycota and the Chytridiomycota lineages splitted first, second and third off of the backbone, respectively with ML bootstrap value 100%, 100%, and 68%, followed by the split of Zoopagomycota, Mucoromycota, Ascomycota and Basidiomycota lineages.

All of the 71 investigated species in the phylogeny were scored based on their multicellular hypha-forming capabilities (Figure 9., Appendix 1.). Applying this scoring we performed ancestral character state reconstruction across the phylogeny to infer the growth mode of fungal ancestors, and the putative origin of hyphal multicellularity and hyphal growth. Results of the reconstruction suggested that hyphal multicellularity evolved from unicellular precursors in some of the earliest fungal ancestors. The distribution of posterior probability (PP) values indicated three ancestral nodes as the most likely origins of hyphal multicellularity, which represent the split of Blastocladiomycota, Chytridiomycota and Zoopagomycota lineages, and are referred hereafter to as BCZ nodes (Figure 9.). The calculated posterior probability values for the multicellular hyphal state started to rise in the most recent common ancestor (MRCA) of the Blastocladiomycota and higher fungi (Chytridiomycota, Zoopagomycota, Ascomycota, Basidiomycota) – PP= 0.53 – and increased to 0.68 and 0.92 in the next two nodes (origin of Chytridiomycota and Zoopagomycota lineages respectively) up in the

fungal backbone. This suggests that multicellular hyphae evolved either in one of the BCZ nodes or that its evolution was a gradual process unfolding in these three nodes. This distribution also reflects the diverse hypha-like structures in the Blastocladiomycota and Chytridiomycota lineages and is consistent with the convergent origins of hypha-like morphologies (Dee et al., 2015; Nagy, 2017; Nagy et al., 2017, 2018).

Figure 9. Phylogenetic relationships among 71 species. Pie charts at nodes show the proportional likelihoods of hyphal (green) and non-hyphal (dark blue) ancestral states reconstructed using Bayesian MCMC. Character states of extant species used in the reconstructions are shown next to species. BCZ nodes: origin of hyphal growth could be assigned with confidence are highlighted (note the uncertainty imposed by filamentous Blastocladiomycota).

4.2. Genome-wide COMPARE analysis of 71 genomes

For performing further analyses on the evolutionary history of putative multicellularity-related gene families, a comprehensive whole genome analysis was

needed. Therefore, using all of the investigated 71 species, COMPARE analysis was performed on whole genomes. Gene family origins and gene duplication/loss histories were reconstructed across all of the gene families in the examined 71 genomes (Figure 10.). The overall pattern revealed by this analysis is that the reconstructed ancestral and extant genome sizes show a big expansion in metazoan species, in Mucoromycota and also in Agaricomycotina.

Figure 10. Reconstructed ancestral genome sizes and gene duplication/loss histories across the 71 genomes. Numbers in dots represent ancestral gene copy numbers, yellow bubbles represent the putative origins (BCZ nodes) of filamentous growth mode. The color codes are as follows: white (UC relatives of animals and fungi), green (MC animals), light grey (MC fungi), dark grey (UC fungi), brownish (ambiguous species) and brown (yeast).

Substantial reduction was observed in gene content at the split of Saccharomycotina and Taphrinomycotina from the Pezizomycotina lineage, resulted in extremely reduced gene content in yeasts. In case of Basidiomycota, the genome reduction is restricted to Ustilaginomycotina, Tremellomycotina and Pucciniomycotina, the three lineages that contain reduced yeast like morphologies similarly to Saccharomycotina.

In the following sections the resulted genome-wide gene duplication/loss catalogue will be analyzed for gene families with previously suggested or novel roles in hyphal multicellularity and hyphal growth.

4.3. No expansion of kinase, receptor and adhesive repertoires in fungi

Cell-to-cell communication and adhesion pathways are key components assumed to be responsible for increased complexity. This concept often correlates with expanded repertoires of genes encoding kinases, receptors and adhesive proteins (de Mendoza et al., 2014; Lim & Pawson, 2010; Suga et al., 2012). One of the most innovative signal transduction system is the phosphotyrosine (pTyr)-based molecular communication system responsible for transmitting and transducing cellular information and used for cell-to-cell coordination in complex organisms. This system evolved recently, around 600 Mya and the evolution of its components was probably indispensable for the emergence of multicellular metazoans from their unicellular ancestors (Lim & Pawson, 2010). A previous study (Suga et al., 2012) investigated the conservation of tyrosine kinases and revealed a difference in gene expansion patterns between cytoplasmic tyrosine kinases (CTKs) and receptor tyrosine kinases (RTKs). This survey suggested that cytoplasmic kinases were already present before the split of filastereans from Metazoa and Choanoflagellata, whereas receptor tyrosine kinases responsible for receiving signals from the environment were highly diversified in each of the different clades and lineages. The metazoan receptor tyrosine kinase repertoire seems to be stable after this diversification and a unique toolkit of these genes retained at the origin of metazoan multicellularity. It is possible that these receptor tyrosine kinases changed their original functions (e.g. receiving environmental signals) and opened the door for novel innovations leading to metazoan multicellularity (Suga et al., 2012).

To investigate whether these kinds of genetic innovations that were found to be key contributors to metazoan MC are also key contributors to hyphal multicellularity, comparative phylogenomic analyses were performed on different signaling system components such as Ser/Thr kinases, hybrid histidine kinases (HHKs), GPCRs and also proteins related to adhesion. COMPARE analyses were performed on the collected genes and their gene families which were identified previously by MCL clustering using whole genomes of the 71 species (Ser/Thr kinases: 954 clusters, hybrid histidine kinases: 96

clusters, GPCRs: 183 clusters, adhesion-related proteins: 23 clusters). To test whether these gene families have undergone diversification at the time of the emergence of filamentous fungi, and might therefore have putative roles in the evolution of hyphal multicellularity and hyphal growth, these analyses focused on the BCZ nodes, and also on the comparison of unicellular and multicellular filamentous fungi. Interestingly, none of the investigated metazoan MC-related functional groups showed significant expansions similar to the patterns in other multicellular lineages such as Metazoa.

The analyses of Ser/Thr kinase gene families in fungal groups revealed that this toolkit was similar in unicellular and hyphal multicellular fungi, with higher kinase diversity found in complex multicellular Agaricomycotina species (Krizsán et al., 2019) and in *Rhizophagus irregularis* also known as *Glomus intraradices* (Gloin1) in Mucoromycota (Figure 11.).

Figure 11. The evolution of Ser/Thr kinases. BCZ nodes (yellow) represent the putative origin of hyphal MC. Bubble size across the tree is proportional to reconstructed ancestral and extant gene copy number. Violin plots for kinases show copy number distribution of gene families in multicellular fungi (grey) and metazoans (green).

Additionally, big contraction was observed in kinase content in yeast-like ascomycetes (Saccharomycotina, Taphrinomycotina) and basidiomycetes (Tremellomycotina and Ustilaginomycotina). Net contractions were inferred in BCZ nodes, from 572 to 529 reconstructed ancestral kinases (81 duplications, 124 losses, Figure 11.), and overall, no substantial kinase gene expansion was observed. Nevertheless, kinase families that expanded here are all contributors of the 3 MAPK pathways in fungi, the mating pheromone (*FUS3*, *KSSI*, *KDX1* in *Saccharomyces cerevisiae*, *MPKB* in *Aspergillus nidulans*), cell wall integrity (*MPKA* in *Aspergillus fumigatus*, *SLT2* in *Saccharomyces cerevisiae*) and osmoregulatory pathways (*HOG1*, *SSK2*, *SSK22* in *Saccharomyces cerevisiae*, *SAKA*, *SSKB* in *Aspergillus nidulans*), all of which indirectly regulate hyphal morphogenesis (Bahn et al., 2007; Biswas et al., 2007; Román et al., 2007). Overall, fungi had fewer Ser/Thr kinases (mean 257) than metazoans (mean 643) and non-fungal opisthokonts (mean 392). These gene numbers seem to be the result of an early expansion of Ser/Thr kinase genes in the LCA of Holomycota and Holozoa.

While signal transduction requirements of metazoan multicellularity have been mostly discussed in the context of receptor tyrosine kinases (RTKs), no evidences were found for domain architectures typical of receptor kinases in fungi. The only group resembling receptor kinases are hybrid histidine kinases (HHK), which include proteins with a sensor domain, a histidine kinase domain and a C-terminal receiver domain that acts as a response regulator. 96 clusters of histidine kinases were identified and analyzed using the same phylogenomic pipeline. Significant expansion (24 duplications, 10 losses) of histidine kinases were inferred in the most recent common ancestor of the Chytridiomycota and other fungi (Figure 12.). The duplicated genes include class III and X histidine kinases, which are associated with fungal developmental programs and regulate essential processes which govern pathogenicity (Defosse et al., 2015; Hérivaux et al., 2016). Deletion of group III hybrid histidine kinases for example caused perturbations in morphogenesis, reduced polarized cell growth and arrested the morphogenetic switch in dimorphic fungal pathogens (Defosse et al., 2015). Deletion of *NIK1/COS-1* HHK genes in *Candida albicans* affected the yeast-hypha transition and virulence (Alex et al., 1998), *drk1* mutant in *Blastomyces dermatitidis* also caused perturbation in dimorphic switching, cell wall integrity and conidiation (Nemecek et al., 2006), and the lack of *Aspergillus fumigatus* HHK gene *NIKA/TCSC* affected hyphal morphogenesis, osmotic stress tolerance, adaptation and conidiation (see other examples in (Defosse et al., 2015)). All of these examples strongly suggest that group III hybrid

histidine kinases are important contributors to hyphal morphogenesis and pathogenicity in filamentous fungi.

Figure 12. The evolution of HHKs. BCZ nodes (yellow) represent the putative origin of hyphal MC. Bubble size across the tree is proportional to reconstructed ancestral and extant gene copy number.

Similarly, class X HHKs also govern morphogenesis and virulence in pathogenic fungi. Studies on CHK1 kinase in *Candida albicans* revealed its importance in virulence, yeast to hypha switch, peroxide adaptation, cell wall composition, quorum sensing, biofilm formation and resistance to antifungals. Also, another class X kinase HHK1 in *Penicillium marneffe* is responsible for the regulation of polarized growth, cell wall biogenesis and sporulation (Defosse et al., 2015; Hérivaux et al., 2016). Another wave of histidine kinase gene expansion was also detected in the MRCA of Mucoromycota and Dikarya with 11 duplications and 4 losses.

In the case of G-protein coupled receptors (GPCRs) an even more extreme difference was observed between fungi and metazoans (Figure 13.). We analyzed 183

GPCR coding gene families, and a large GPCR expansion was observed in animals. This expansion started at the MRCA of metazoa with 56 ancestral gene copy numbers and resulted in 117-531 genes in extant animals. Also, out of the analyzed GPCR families, only 19 were characteristic to fungi and only one of them was generally conserved across most of the fungal species. This family contains STE3 and STE3-like pheromone receptors of fungi (STE3 in *Candida albicans* and *Saccharomyces cerevisiae*, PREA in *Aspergillus fumigatus*), originates at the MRCA of Dikarya and Mucoromycota, and shows significant expansion in the Agaricomycotina lineage. Members of the STE3 family are class II GPCRs, they are usually involved in pheromone-dependent signal transduction, cellular conjugation and cell fusion.

Figure 13. The evolution of GPCRs. BCZ nodes (yellow) represent the putative origin of hyphal MC. Bubble size across the tree is proportional to reconstructed ancestral and extant gene copy number. Violin plots for receptors show copy number distribution of gene families in multicellular fungi (grey) and metazoans (green).

STE3 GPCRs are expressed in alpha cells of *Saccharomyces cerevisiae*, and they are responsible for sensing the mating pheromone ‘a’ factor (Xue et al., 2008). Due to their diversification in fruiting body forming fungi, we can speculate that this gene family can be related to complexity in some extent, but functional studies have not been confirmed this hypothesis yet.

Adhesive cell surface proteins are key mediators of the transition to multicellularity in colonial and aggregative lineages (Abedin & King, 2010; Brunet & King, 2017; Seb-Pedrs et al., 2017), which is reflected in their higher gene copy numbers in multicellular organisms (Kirk, 2005). We identified 23 families of putative adhesion-related proteins in fungi, including adhesins, flocculins, hydrophobins, various lectins and glycosylphosphatidylinositol (GPI)-anchored cell wall proteins. Evolutionary reconstructions of these gene families did not reveal significant number of gene duplications in relation to hyphal multicellularity, but only a small net contraction (from 17 to 14 copies) in BCZ nodes.

Figure 14. The evolution of adhesion-related genes. BCZ nodes (yellow) represent the putative origin of hyphal MC. Bubble size across the tree is proportional to reconstructed ancestral and extant gene copy number.

Ancestral gene copy numbers of adhesion-related genes remained more or less similar (up to 14 before the split of Dikarya) along the fungal backbone, with notable expansions observed later, in Agaricomycotina and Saccharomycotina yeast-like fungi (*Candida albicans*, *Pichiastipitis*, *Debaryomyces hansenii*) (Figure 14.).

The lack of a notable expansion in BCZ nodes probably reflects a marginal role of these adhesion-related proteins in the evolution of early hyphae, but potentially also the scarcity of adhesion proteins annotated in early-diverging fungi, or the effects of sequence divergence. The high gene expansion in the Agaricomycotina lineage was mainly driven by class1 hydrophobins and homologs of the *Cryptococcus neoformans* *CFL1* gene. CFL1 is an adhesive protein with roles in signaling and morphotype transition between yeast and filamentous states. It orchestrates cell adhesion, filamentation and also virulence, acting downstream of ZNF2, which is an important transcription factor, required for cell surface modification during morphogenesis and pathogenesis (Wang et al., 2012). Diversification of these gene families coincides with the ability to produce complex, multicellular fruiting bodies and is probably attributable to the emergence of complex multicellularity in the Agaricomycotina lineage (Nagy et al., 2018). The higher gene copy numbers in yeast species relate to several yeast-specific adhesin and lectin-like cell wall proteins, such as the IFF4 or the HYR1 GPI-anchor cell surface proteins or the PGA62/FLO1, that were experimentally characterized in human pathogenic fungi (e.g. *Candida albicans*) (Kempf et al., 2009; Moreno-Ruiz et al., 2009).

Taken together, the evolution of Ser/Thr kinase, histidine kinase, GPCR and adhesive protein repertoires highlight an important difference between fungi and multicellular metazoans. During the evolutionary analyses of these gene families we observed no significant expansions in filamentous fungi, whereas Ser/Thr kinase and adhesion related genes expanded in complex multicellular Agaricomycotina. This phenomenon might be explained by the two-step nature of the evolution of complex multicellularity in fungi (Nagy et al., 2017, 2018) that proceeds through an intermediate complexity level, which can be the hyphal multicellularity, as opposed to metazoans, where complex multicellularity evolved in a more direct way (Niklas et al., 2013; Niklas & Newman, 2013). The observation that these 'classic' gene families responsible for multicellular metazoan evolution can't explain the evolution of hyphal multicellularity, prompted us to examine other gene families, whose evolution might show a better correlation with that of hyphae.

4.4. Evolution of hypha morphogenesis genes

A dataset of hypha morphogenesis-related genes was created to address if changes in such gene families during evolution correlate with the emergence of multicellular hyphae. 651 hypha morphogenesis-related genes were identified, which belong to 362 gene families based on the MCL clustering of the 71 genomes (Appendix File 1.). These genes were collected from 519 publications, covering our current knowledge on hyphal morphogenesis, mostly from well-studied fungal model systems such as *Aspergillus fumigatus*, *Aspergillus nidulans*, *Neurospora crassa*, *Saccharomyces cerevisiae*, *Candida albicans*, *Ustilago maydis* etc. (see the complete list of the 651 genes in Appendix File 2.). The collected genes were subsequently categorized into 10 functional groups according to the broader function they fulfill in filamentous hyphal growth. The 10 functional groups are as follows: “actin cytoskeleton regulation”, “polarity establishment and maintenance”, “cell wall biogenesis and remodeling”, “septation” (including septal plugging), “signaling”, “transcriptional regulation”, “vesicle transport”, “microtubule-based transport”, “cell cycle regulation” and “adhesion” (see the detailed analysis of adhesion-related gene families in the previous chapter) categories. The two categories “polarity establishment and maintenance” and “vesicle transport” contained the largest number of genes (107 in each), whereas the “cell cycle regulation” category contained the fewest (11) (Figure 15.).

Figure 15. Distribution of literature-collected hypha morphogenesis-related genes among 10 main functional categories.

Origins and evolutionary dynamics of gene families in the 10 functional categories were reconstructed by calculating gene duplication and loss events across the species phylogeny, using the COMPARE pipeline. We examined multiple types of evolutionary innovations to identify the most important mechanisms underlying the emergence of

hyphal growth. To account for uncertainty in the exact origin of hyphae, the analyses of hypha morphogenesis genes focused on three different nodes (BCZ nodes).

4.4.1. Actin cytoskeleton

In the “actin cytoskeleton” category (Figure 16.) 55 genes were identified and grouped into 35 gene families based on MCL clustering. The analysis of these gene families revealed big lineage-specific gene expansion in higher animals (*Drosophila melanogaster*, *Caenorhabditis elegans*), possibly due to their big importance in the development of complex multicellularity in Metazoa. Compared to animals, no gene family expansion was detected in multicellular fungi. Also, there was no big difference between gene copy numbers in hyphal multicellular and unicellular fungi.

Further, reduction was observed in gene copy numbers in Tremellomycetes, Ustilaginomycotina and Pucciniomycotina lineages and relatively small reduction in Agaricomycotina species compared to other fungi. After the split of Saccharomycotina and Pezizomycotina a gradual gene loss was observed along the Pezizomycotina lineage. Interestingly, yeast like fungi did not show the same pattern in case of actin cytoskeleton-related genes, Ascomycota yeasts did not show reduction in gene copy numbers compared to Basidiomycota yeasts, where severe contraction was observed. But difference was observed not only among the yeast-like species of these two groups, but also in gene copy numbers between Ascomycota and Basidiomycota, in general (Figure 16.).

Analyzing gene family origins of actin cytoskeleton-related genes, we found that many of the investigated gene families – 17 out of the 35 (48%) – were conserved across all eukaryotes. This conservation can be due to their universal importance and roles in basic cellular functions such as cytoskeletal remodeling and endocytosis. Beside their ancient origins and basic cellular functions, these genes also play important roles in polarized growth and hyphal morphogenesis. For example, fimbrins (FIMA/SAC6 in *Ashbya gossypii*, SAC6 in *Aspergillus fumigatus*) are actin-bundling proteins, having roles in the organization and maintenance of the actin cytoskeleton, therefore, they are required for endocytosis and fast polarized growth (Jorde et al., 2011). The ARP2/3-complex (actin cytoskeleton-regulatory complex) proteins (ARP3 in *Neurospora crassa*) and their activators, the Wiskott-Aldrich Syndrome Proteins (WASPs) (LAS17 in *Ashbya gossypii*) are also deeply conserved, they are necessary for proper actin patch organization and they support hyphal growth in hypha-forming fungi (Philippsen et al., 2005).

Figure 16. Ancestral reconstruction of gene copy numbers in “actin cytoskeleton” category. Bubble size is proportional to reconstructed ancestral gene copy number. P-values of enrichment of duplications are shown in Table 2. (Fisher’s exact test, FDR correction).

This set of conserved genes also contains profilin and coronin protein coding genes, all of them are important patch component and cytoskeletal modulator. These actin binding proteins, beside their roles in filament turnover and actin cytoskeleton remodeling, indirectly regulate and govern hyphal growth. Focusing on BCZ nodes (the putative origins of hyphal growth), the gene duplication and loss analysis revealed further gene families that originated earlier during evolution but also show duplications at the origin of hyphal growth. But beside only a few duplications, no significant expansion was observed in BCZ nodes. Those gene families that showed duplications in BCZ code for example the WAL1 protein in *Candida albicans*, which is a Wiskott-Aldrich syndrome protein (WASP) homolog. This protein is required for the organization of the actin cytoskeleton, endocytosis, vacuole morphology and important in hyphal morphogenesis

(Walther & Wendland, 2004). Another protein in *Candida albicans*, the END3 component of the PAN1 actin cytoskeleton-regulatory complex, and its gene family also show duplications in BCZ nodes. This protein is required for the internalization of endosomes during actin-coupled endocytosis (Epp et al., 2013).

The most important genes and gene families coming from this analysis are those ones whose appearance mapped to the BCZ nodes (Table 1.). These gene families originated at the time of the emergence of multicellular hyphae and are conserved afterwards, and therefore we can assume that these genes and gene families can be key contributors to the evolution of hyphal morphogenesis. SLM1 and its homolog SLM2 in *Saccharomyces cerevisiae* are pleckstrin homology domain-containing proteins. These proteins are critical effectors of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) which is a key second messenger of PI(4,5)P₂ signaling, that is required for actin cytoskeleton and membrane dynamics regulation, among others. These proteins integrate inputs not only from PI(4,5)P₂ signaling but also from the TOR2 pathway via the interaction with the TORC2 signaling complex (Fadri et al., 2005). These interactions result in downstream responses which are essential for cell growth and actin cytoskeleton polarization and probably for polarized hyphal growth in filamentous fungi (*SLM1* and *SLM2* orthologs in filamentous fungi await further characterization). Another protein originated in the BCZ nodes is the PAN1 scaffolding protein in *Candida albicans*, a component of the PAN1 actin cytoskeleton regulatory complex. This protein is required for the internalization of endosomes during actin-coupled endocytosis by linking the site of endocytosis to the cell membrane-associated actin cytoskeleton. Overexpression of *PAN1* induces strong hyphal morphogenesis and cells exhibit higher hyphal specific gene expression, emphasizing the importance of endocytosis in hyphal morphogenesis and assuming connections between endocytic processes and signal transduction pathways of hyphal induction (Bar-Yosef et al., 2018; Kornitzer, 2019). SLA1 (in *Saccharomyces cerevisiae* and *Candida albicans*), another protein originated in BCZ nodes is also an important component of the actin cytoskeleton-regulatory complex, it localizes at the hyphal tip and actin patches that form the sites of endocytosis. Its deletion reduces the number of cortical actin patches and causes decreased polarized growth rate during hypha formation (Reijntjes et al., 2010).

4.4.2. Cell cycle

“Cell cycle” category (Figure 17.) contained 11 collected genes (5 gene families) related to hyphal morphogenesis. Gene family expansion was observed in *Thecamonas trahens*, higher animals, early diverging fungi (*Allomyces*, *Spizellomyces*, *Gonapodya*) and in several Saccharomycotina yeasts, whereas family reduction was found in Pezizomycotina and Basidiomycota lineages. Two families – containing mitotic cyclins and kinases – have ancient eukaryotic origins, whereas two other families originated at the MRCA of Saccharomycotina.

Figure 17. Ancestral reconstruction of gene copy numbers in “cell cycle” regulation category. Bubble size is proportional to reconstructed ancestral gene copy number. P-values of enrichment of duplications are shown in Table 2. (Fisher’s exact test, FDR correction).

One of the ancient gene families showed duplications in BCZ nodes. This family contains the CLB2 mitotic CDK1 cyclin, which is required for wild-type mitotic exit and has a

role in cell polarization in *Saccharomyces cerevisiae* and *Candida albicans* (Bensen et al., 2005). Out of the 5 gene families, only one family originated at the BCZ nodes and was conserved thereafter. A known member of this family is the *WHI2* gene from *Saccharomyces cerevisiae* which is required for cell cycle arrest upon carbon source depletion. Overexpression of *WHI2* stimulates filamentous growth by the reorganization of the cell cycle and changing the budding pattern from axial to polar. Other notable consequence of *WHI2* overexpression is the incomplete cytokinesis (Radcliffe et al., 1997).

4.4.3. Cell wall biogenesis and remodeling

We collected 94 genes (40 gene families) in the “cell wall biogenesis and remodeling” category (Figure 18.). Analysis of gene copy number variation between extant unicellular and multicellular fungi did not reveal any remarkable difference. Compared to the “actin cytoskeleton” and “cell cycle” categories, only a small part of the cell wall-related gene families had ancient eukaryotic origin (8 families out of the 40, 20%). However, a big expansion of these gene families was found at the MRCA of fungi and metazoa and after at the MRCA of fungi. Beside these early gene family expansions, we also found significant expansion in the BCZ nodes. The reconstructed ancestral gene copy numbers gradually increased along the backbone (89 to 101 to 109 gene copy numbers). Sixteen gene families (40%) out of the 40 contributed to these expansions in BCZ nodes. The class V chitin synthase (*CHSE* in *Aspergillus fumigatus*) containing gene family showed the highest duplication number (8). CHSE chitin synthase is required for polarized hyphal growth, conidiation and normal conidiophore development. Mutant strains have reduced levels of mycelial chitin (Aufauvre-Brown et al., 1997). The class I and class IV chitin synthase gene families also showed duplications (5 and 6 respectively) in BCZ nodes. CHSF has similar role in hyphal growth, it is crucial for hyphal extension and normal morphology, whereas CHSA in *Aspergillus fumigatus* seems to be not essential in chitin synthesis and cell wall stress (Rogg et al., 2011). Class III chitinases also duplicated at BCZ nodes. CHIA1 is a GPI-anchored chitinase in *Aspergillus fumigatus* and it is localized at the apex of germ tubes and hyphal branching sites. Mutant strains exhibit a decreased rate of germination of conidia and a lower hyphal growth rate (Yamazaki et al., 2008). Other relevant families that showed duplications in BCZ nodes contain for example the FKS1 1,3-beta-D-glucan synthase, the EXG1 exo-1,3-beta-

glucanase, ORLA trehalose 6-phosphate phosphatase and the PMT4 O-mannosyltransferase in *Aspergillus fumigatus*. All of these families are key contributors to the normal cell wall biosynthesis and hyphal growth. Beside the big number of these cell wall-related gene families that show duplications in BCZ nodes, this category contains the biggest number of those gene families that originated in BCZ nodes (6 gene families (35%) mapped to BCZ nodes out of the total 17 families, Table 1.).

Figure 18. Ancestral reconstruction of gene copy numbers in “cell wall biogenesis and remodeling” category. Bubble size is proportional to reconstructed ancestral gene copy number. P-values of enrichment of duplications are shown in Table 2. (Fisher’s exact test, FDR correction).

The CRH3 (in *Aspergillus fumigatus*) and UTR2 (in *Saccharomyces cerevisiae*) encoding gene family consists of chitin transglycosylases. These proteins mediate the linkage between chitin and glucan chains by transferring the chitin to beta-1,6 and beta-1,3 glucans. Also, they localized to the sites of polarized growth and control morphogenesis via the remodeling of fungal cell wall in response to cell wall stress (Arroyo et al., 2016). Another family mapped to BCZ nodes contains beta-1,3 glucanoyltransferases, GEL7

from *Aspergillus fumigatus* and GAS1 from *Saccharomyces cerevisiae*. These glucanosyltransferases are involved in cell wall remodeling during fungal germination or hyphal branching (Zhao et al., 2014). Families of CHSD class VI chitin synthase from *Aspergillus fumigatus* (Muszkieta et al., 2014), the *Neurospora crassa* CPS1 polysaccharide synthase (Fu et al., 2014) and the DFG5 from *Candida albicans* also mapped to BCZ nodes. DFG5 is a GPI-anchored cell surface protein. Being as a potential signal transducer, it has an important role in hypha development by capturing and transmitting external signals required for hypha-specific gene expression. For example, *DFG5* mutation affects the expression pattern of hypha-specific gene *HWPI*. This connection between cell wall development and hyphal growth (see knock-out studies of other cell wall-related genes that affect hyphal morphogenesis in (Lussier et al., 1998; Navarro-García et al., 1998; Richard et al., 2002)) can be the result of a possible cell wall integrity “checkpoint” that is required for hyphal morphogenesis. This “checkpoint” can be a possible explanation of the structural impairment accompanied by regulatory responses that affect hypha-specific gene expression (Spreghini et al., 2003). The last family mapped to BCZ nodes contains the KRE6 beta-1,6-glucan synthase from *Candida albicans*. Deletion of *KRE6* caused slow growth and 50% reduction of glucan content and abnormal cell wall structure, and defects in cell separation. Also, the simultaneous deletion of *KRE6* and its homolog *SKNI* completely abolished filamentous growth. Expression studies on *KRE6* and *SKNI* revealed that *KRE6* is constitutively expressed and *SKNI* expression is activated upon hyphal induction (Han et al., 2019). Although the two homologs are functionally redundant in cell wall biogenesis, these results indicate their different roles in hyphal morphogenesis.

4.4.4. Microtubular transport

In the „microtubular transport” category (Figure 19.), 32 hypha morphogenesis-related genes (19 gene families) were collected. The difference between gene copy numbers of hyphal multicellular and unicellular fungi was significant. On average, unicellular fungi have higher copy numbers of these gene families than Mucoromycota, Ascomycota and many Basidiomycota fungi. Gene family expansion was observed in the MRCA of metazoa and fungi, in several Agaricomycotina fungi, and also in node B. We found severe contractions in Saccharomycotina, Taphrinomycotina and in Basidiomycota

yeasts. The conservation of gene families across species was 37%, 7 families out of the total 19 were conserved across all eukaryotes.

Figure 19. Ancestral reconstruction of gene copy numbers in “microtubular transport” category. Bubble size is proportional to reconstructed ancestral gene copy number. P-values of enrichment of duplications are shown in Table 2. (Fisher’s exact test, FDR correction).

The expansion was restricted to 6 families in BCZ nodes. The biggest number of gene duplication (6) was found in the NUDF (*Aspergillus nidulans*), PAC1 (*Saccharomyces cerevisiae*) and LIS1 (*Neurospora crassa*) containing family. These are microtubule-plus-end binding proteins which have strong association with the dynein/dynactin complex and microtubule network organization. They are required for nuclear and organelle migration during cell growth and hypha elongation. Deletion of *LIS1* and *LIS2* resulted in poor hyphal growth and reduced conidiation (Callejas-Negrete et al., 2015). Three duplications were mapped to BCZ nodes in kinesin (KIN1, KIN14 in *Ustilago maydis*) encoding family. These motor proteins participate in long-range vesicle and

organelle transport. In the filamentous hyphal forms of *Ustilago maydis*, the MT plus-ends are focused to the hyphal tip, therefore kinesin motors can be key components of polarized growth. Deletion of *KIN1* and *KIN3* cause bipolar growth pattern and short filaments, whereas in normal circumstances they are upregulated in hyphae (Schuchardt et al., 2005). The remaining 4 families contain EB1 MT plus-end binding protein (*Aspergillus fumigatus*), MIPA gamma-tubulin (*Aspergillus nidulans*) and NUDA dynein (*Aspergillus nidulans*) coding gene families having 2, 1 and 1 duplications respectively at BCZ nodes. Gene families originated in BCZ nodes were not found.

4.4.5. Polarity establishment and maintenance

107 genes (63 gene families) were collected in “polarity establishment and maintenance” category (Figure 20.). No significant difference was found between gene copy numbers of hyphal multicellular and unicellular fungi. Gene family expansion (from 86 to 106 genes) was detected at the MRCA of fungi and metazoans, in BCZ nodes (from 102 to 114 genes), in Agaricomycotina and in Mucoromycota lineages. Significant gene copy number difference was observed between metazoans and their unicellular relatives, indicating the importance of these polarity-related gene families in the evolution of metazoan complexity. Unfortunately, this kind of difference between unicellular and multicellular fungi was not found. Basidiomycota yeasts (*Cryptococcus neoformans*, *Ustilago maydis*, *Malassezia globosa*, *Rhodospiridium toruloides*, *Sporobolomyces roseus*) and *Wallemia sebi* showed extreme gene copy number reduction. 29% (18 out of the 63) of gene families was conserved and mapped to the MRCA of Eukaryotes. An increment in ancestral gene copy numbers (95 to 102 to 114) was detected in BCZ nodes, coming from the expansion of different polarity-related gene families. Seven duplications were found in RACA, RHO4 (*Aspergillus fumigatus*), MODA (*Aspergillus nidulans*), RHO1-4 (*Candida albicans*) and CDC42 (*Saccharomyces cerevisiae*) encoding gene family. These RHO-type GTPases are the members of the RAC-CDC42-CDC24 GTPase module and they required for polarized growth and hyphal morphogenesis. They organize the morphogenetic machinery and enable polarity axis formation and stabilization by the reorganization of cytoskeletal network and by the recruitment of polarity markers (polarisome components, cell-end markers etc). These proteins have diverse roles and they act on multiple levels. They regulate the polarization of actin cables and patches to the apex, the formation of exocyst complex, protein secretion, actin ring formation at the

site of septation and the activation of different signaling networks (for example the CDC42 activates MAPK module through STE20 during pseudohypha formation) (Johnson, 1999). Another family containing *YCK2* (*Candida albicans*) shows 4 duplications in BCZ nodes. YCK2 is a plasma membrane protein with multifunctional kinase activity. Loss-of-function mutants are hyperpolarized and display defects in cellular morphogenesis, cytokinesis and endocytosis, suggesting a role in the regulation of polarized cell growth, cell wall integrity, biofilm formation and virulence in *Candida albicans* (Jung et al., 2017; Robinson et al., 1999). The Ras gene family that contains *RASA* from *Aspergillus fumigatus* showed 2 duplications at BCZ nodes. RASA is a conserved GTPase protein, known as a main regulator of hyphal growth and development, also linked to cell wall integrity. It localizes primarily to the plasma membrane of actively growing hyphae (Fortwendel et al., 2012). The ROM2 (*Aspergillus fumigatus*) coding family also has 2 duplications, which mapped to BCZ nodes. Members of this family are guanine nucleotide (GDP/GTP) exchange factors (GEF) for RHO proteins. Despite their low duplication numbers in BCZ nodes, it is worth to mention three additional polarity-related gene families. Family of LAG1 consists of ceramide synthases which are important components of polarized growth (Li et al., 2006). The lack of *LAG1* gene in *Candida albicans* caused severe defects in growth rate and hyphal morphogenesis, but its overexpression induced pseudohyphal growth, suggesting that *lag1* is necessary for relaying signals that induce hypha-specific gene expression (Cheon et al., 2012). PI4P 5-kinases are responsible for the formation of PI4,5P2 pools that control polarized growth and septation. Polarized growth of *Neurospora crassa* requires the formation of PtdIns(4,5)P2 by the PI4P 5-kinase MSS4 at the hyphal tip (Mähs et al., 2012). In *Saccharomyces cerevisiae* MSS4 drives the cascade of PI4,5P2 - ROM2 - RHO1/2 - PKC1/BNI1 or PI4,5P2 - SLM1/2 which finally regulates the actin cytoskeleton and polarized growth (Levin, 2005). Interestingly, all families of these network components show duplications or originated in BCZ nodes, at the putative origin of hyphal growth. Besides the aforementioned gene families, we also found three polarity-related gene families that appeared in BCZ nodes. The first one encodes BNI-1 (*Neurospora crassa*) and Afu6g04940 (SEPA homolog in *Aspergillus fumigatus*) polarisome components, having role in polarity establishment and maintenance and septum formation.

Figure 20. Ancestral reconstruction of gene copy numbers in “polarity” category. Bubble size is proportional to reconstructed ancestral gene copy number. P-values of enrichment of duplications are shown in Table 2. (Fisher’s exact test, FDR correction).

These polarisome proteins mediate the actin cable assembly in filamentous fungi and have roles in various morphogenetic processes, like the organization of the actin cytoskeleton at the sites of localized cell wall deposition, the formation of actin ring at septation sites and generation of a stable polarity axis of polarized hyphal growth. The BNI1 formin exhibits a dynamic localization pattern at the apical cap and septation sites, and along with other polarisome components, it is required to establish and maintain the Spitzenkörper and organize the tip growth apparatus. *Bni1* and *sepA* mutants are unable to form actin cables, exhibit polarity and septation defects (Harris et al., 1997; Lichius et al., 2012). Another family mapped to BCZ nodes contains additional formins (BEM1 (*Saccharomyces cerevisiae*), BEMA (*Aspergillus nidulans*) and Afu4g04120 (*Aspergillus fumigatus*)). These SH3-domain containing, actin cytoskeleton reorganizing factors are likewise involved in establishing cell polarity and morphogenesis via functioning as a scaffold protein for other polarity complexes that include CDC24, STE5,

STE20 and RSR1 (Chenevert et al., 1992). BEMA is also required for Spitzenkörper formation and the localization of SEPA, which is another protein mapped to BCZ nodes (Leeder & Turner, 2008). *RGSB* (*Aspergillus fumigatus*) and its ortholog in *Saccharomyces cerevisiae*, *RAX1* are also main contributors to polarity establishment and maintenance, and their gene family also originated in BCZ nodes. RAX1 is an N-glycosylated protein, specifically required for the establishment of bipolar budding pattern in yeast (Fujita et al., 2004). Its deletion causes reversion to axial budding pattern. In filamentous fungi, RAX1 orthologs control vegetative growth and conidiation (Igbalajobi et al., 2017; Lafon et al., 2006).

4.4.6. Septum formation

In „septum formation” category (Figure 21.) we collected 58 genes (33 gene families) related to hyphal morphogenesis. Gene copy numbers of the collected gene families were surprisingly bigger in extant unicellular than in hyphal multicellular fungi, except *Rozella allomyces*, which has lower gene copy numbers. Gene family expansion was found in Metazoa and Mucoromycota whereas Basidiomycota and Ascomycota yeasts showed reduction in gene copy numbers. Fifteen gene families (45%) out of the 33 were conserved across all eukaryotes. Focusing on BCZ nodes in “septation” category, we found expansions in 7 gene families. Five duplications were mapped to BCZ nodes in the KIC1 (*Saccharomyces cerevisiae*) and Afu5g02220 (*Aspergillus fumigatus*) protein kinase coding PAK/STE20 gene family. The serine-threonine kinase KIC1 is part of the RAM network and in collaboration with CBK1, MOB2, TAO3 and HYM1, it regulates ACE2 transcription factor, cellular polarity and morphogenesis. Additionally, KIC1 is required for cell wall integrity and physically interacts with CDC31 centrin, which is a component of the spindle pole body. The deletion of *KIC1* and other RAM component coding genes causes round cell morphology and cell separation defects (Nelson et al., 2003). The TAO3 coding gene family also showed duplications in BCZ nodes. TAO3 is another component of the RAM signaling network, and therefore, similarly to KIC1, it is involved in the regulation of ACE2, a transcription factor that controls cell separation genes in *Saccharomyces cerevisiae*. Beside the regulation of the ACE2 TF, the RAM protein kinase signaling network regulates a wide range of cellular processes, like cell wall integrity, mating, polarized growth and also pathogenesis in filamentous fungi (Saputo et al., 2012). The analysis of the septin family also revealed 2 duplications here.

Figure 21. Ancestral reconstruction of gene copy numbers in “septation” category. Bubble size is proportional to reconstructed ancestral gene copy number. P-values of enrichment of duplications are shown in Table 2. (Fisher’s exact test, FDR correction).

This family contains the main contributors to septin ring formation during cytokinesis. These components are the CDC10 in *Ashbya gossypii*, CDC11 in *Saccharomyces cerevisiae*, CDC3, CDC10 and CDC12 in *Candida albicans* and ASPA, ASPB, ASPC and ASPE from *Aspergillus fumigatus*. All of these family members have roles in localized chitin deposition in cell wall, septation, bud site selection, cell cycle control, cytokinesis, formation of mating projections, regulation of branching pattern and sporulation (Ford & Pringle, 1991; González-Novo et al., 2008; Lindsey et al., 2010). Beside septins, additional gene families duplicated in BCZ nodes, that code for example endo-beta-1,3-glucanases (ENGL1 in *Aspergillus fumigatus* and ENG1 in *Saccharomyces cerevisiae*) and CYK3 (*Neurospora crassa*). In “septation” category, we did not find any gene families emerging from BCZ nodes.

4.4.7. Signaling

In „signaling” category (Figure 22.) 89 genes (46 gene families) were collected. Significant difference between gene copy numbers of hyphal multicellular and unicellular fungi was not found. Gradual gene family expansion was observed in BCZ nodes, first from 156 to 164 (in node B) and after from 164 to 178 (in node C) copy numbers. Additional expansions were found in Agaricomycotina and Mucoromycota fungi and also in animals. Out of the 46 gene families 16 (35%) were conserved, 18 (39%) showed expansions in BCZ nodes and 2 families were mapped to the putative origin of hyphal growth. The SSY1 (*Saccharomyces cerevisiae*) amino acid sensor encoding gene family had 7 duplications and also appeared at the BCZ nodes. The amino acid concentration usually affects the filamentous growth of yeasts. The SSY1 amino acid permease is a member of the SSY1–PTR3–SSY5 (SPS) plasma membrane amino acid sensor system, senses the external amino acid concentration and transmits intracellular signals. This signaling process results in the regulation of amino acid permease genes. Loss-of-function mutations in genes related to these signaling processes induce elongation and enhance invasive growth in yeast (Klasson et al., 1999; Palecek et al., 2002). The ortholog of SSY1 in *Candida albicans* is required for wild-type hyphal growth, but the role of this amino acid permease protein family in filamentous fungi is not known. Another gene family originated in BCZ nodes encodes RICA (*Aspergillus fumigatus*) and RIC8 (*Fusarium gramineis*). These GDP/GTP exchange factors regulate G proteins that function upstream of the cAMP-PKA signaling pathway and play important roles in fungal development, virulence and secondary metabolism (Wu et al., 2015). Additionally, seven duplications were found in BCZ nodes in the case of a serine/threonine protein kinase-encoding gene family, having the SKS1 (*Saccharomyces cerevisiae*). Not only the amino acid signaling, but also glucose sensing regulates pseudohypha formation in yeast. SKS1 kinase is an important regulator of filamentation and glucose/nitrogen signaling. It is involved in the adaptation to low concentrations of glucose and nitrogen by mediating cellular responses to glucose and nitrogen limitation through the GPR1 nutrient sensor and the cAMP-dependent PKA pathway that regulates pseudohyphal differentiation (Johnson et al., 2014). Four duplications were found in another important gene family related to signaling. This family encodes PTCG (*Aspergillus fumigatus*) (Winkelströter et al., 2015) and its orthologs PTC2 in *Candida albicans* (Gil-Bona et al., 2015) and PTC2 in *Saccharomyces cerevisiae* (Young et al., 2002).

Figure 22. Ancestral reconstruction of gene copy numbers in “signaling” category. Bubble size is proportional to reconstructed ancestral gene copy number. P-values of enrichment of duplications are shown in Table 2. (Fisher’s exact test, FDR correction).

These HOG1 phosphatases regulate the activation of the osmotic stress pathway (HOG pathway) and also have effects on cell surface modifications, conidiation, germination, adhesion, biofilm formation and universally on cell wall integrity (CWI) pathway. Null mutants usually have impaired CWI, biofilm formation and virulence, and they have increased sensitivity to oxidative and osmotic stresses. Four-four duplications were found in BCZ nodes in MEP2 and BMH1-BMH2 (*Saccharomyces cerevisiae*) encoding gene families. The ammonium permease MEP2 (MEAA in *Aspergillus fumigatus*) is a nitrogen compound detecting receptor and membrane transporter, having role in the regulation of pseudohyphal growth, functioning upstream of GPA2, RAS2 and cAMP. Yeast cells lacking this receptor type can acquire ammonium and grow at normal rates, but they are incapable to form filaments in response to ammonium limitation (Lorenz & Heitman, 1998). BMH1 and BMH2 (ARTA in *Aspergillus fumigatus*) are other regulators of pseudohypha formation in yeast. In general, 14-3-3 proteins are conserved eukaryotic

regulators involved in diverse cell functions, such as exocytosis, vesicle transport, RAS/MAPK signaling and cell cycle-related processes. The BMH1 and BMH2 14-3-3 proteins are essential for pseudohyphae formation by fulfilling their roles in RAS/MAPK cascade signaling network, for example via their interaction with the STE20 kinase (Roberts et al., 1997). The family having SAKA (*Aspergillus fumigatus*) MAP kinase orthologs showed 2 duplications in BCZ nodes. These kinases (HOGA in *Aspergillus nidulans*, HOG1 in *Saccharomyces cerevisiae*, OS-2 in *Neurospora crassa* etc.) share a conserved role in osmotic and oxidative stress responses and also play role in regulating apical cell growth (Han & Prade, 2002; Ma & Li, 2013; Krantz et al., 2006). SHO1 (*Aspergillus fumigatus*) transmembrane osmosensor is another HOG MAPK pathway component (Yang et al., 2011). Its family has 2 duplications in BCZ nodes. SHO1 orthologs are required for filamentous hyphal growth, conidial germination, actin distribution and the response to oxidative stress. SHO1 in *Saccharomyces cerevisiae* regulates pseudohyphal growth together with MSB2, and cooperates with STE20 and STE50. In *Candida albicans*, *sho1* mutants show defects in cell wall biogenesis and morphogenesis, whereas in *Aspergillus fumigatus* deletion of *SHO1* causes reduced radical hyphal growth. In “signaling” category another 10 family were found with 1-1 duplication in BCZ nodes encoding SITA, GPAA, PKAR, ACYA, RICA from *Aspergillus fumigatus*, RIM20, STE11, SLN1, NPR3 from *Saccharomyces cerevisiae* and SSK1 from *Candida albicans* (see their detailed descriptions in Appendix File 2.).

4.4.8. Transcriptional regulation

In „transcriptional regulation” category (Figure 23.) 53 genes (41 gene families) were collected. Only 2 (5%) gene families were conserved across all eukaryotes. Gradual expansion in gene copy numbers was found first at the MRCA of metazoa and fungi (from 8 to 18) and after in BCZ nodes (expansion from 20 to 31 to 36 to 42 respectively). Four duplications were found in BCZ nodes in a family which contains Afu6g04490, a putative cAMP-independent regulatory protein (an ortholog of OSAA (Orchestrator of Sex and Asex) in *Aspergillus fumigatus*, MIT1 in *Saccharomyces cerevisiae*, WOR1 in *Candida albicans*). These WOPR domain containing proteins are fungal-specific DNA-binding transcription factors (Cain et al., 2012) responsible for different morphological changes in different species. OSAA in *Aspergillus nidulans* regulates morphological development and spore formation (Alkahyyat et al., 2015). MIT1 in *Saccharomyces cerevisiae* is a

central transcription factor that drives filamentous growth by binding other transcription factors responsible for the regulation of hyphal growth. WOR1, a MIT1 ortholog in *Candida albicans* is a core regulator of the white-opaque phenotypic switch and adhesion. A gene family encoding SIN3 in *Candida albicans* also showed 4 duplications in BCZ nodes. SIN3 is a component of a specific histone deacetylase complex that binds to the promoter of a bHLH (basic helix-loop-helix) transcription factor EFG1, which is a regulator of morphogenesis in *Candida albicans*. *Sin3* mutants show budding pseudohyphal morphology and unable to form true hyphae (Tebarth et al., 2003). Out of the investigated 41 gene families, 2 originated in BCZ nodes.

Figure 23. Ancestral reconstruction of gene copy numbers in “transcriptional regulation” category.

Bubble size is proportional to reconstructed ancestral gene copy number. P-values of enrichment of duplications are shown in Table 2. (Fisher’s exact test, FDR correction).

The first one encodes APSES transcription factors (Zhao et al., 2015), key regulators of fungal development, asexual sporulation, mating, cellular differentiation, filamentous growth, secondary metabolism and also virulence. STUA (*Aspergillus fumigatus*) is one of these APSES transcription factors. Being as a developmental modifier, it regulates

morphogenesis and control the central transcription factor cascade involved in the regulation of conidiophore development (Harris et al., 2009; Miller et al., 1992; Sheppard et al., 2005). Its ortholog in *Saccharomyces cerevisiae*, the PHD1, on the other hand mediates the transition from spherical growth to tubular growth (Gimeno & Fink, 1994). It is an inducer of FLO11, a "pseudohyphal determinant" and its overexpression induces invasive pseudohyphal growth on rich medium. Interestingly, SOK2, a paralog of PHD1 in *Saccharomyces cerevisiae* has an antagonistic function, it represses *PHD1* and acts downstream of cAMP-dependent PKA pathway to negatively regulate the pseudohyphal growth (Ward et al., 1995). EFG1 ("enhanced filamentous growth protein 1") is another APSES family member in *Candida albicans*. This transcription factor is a positive regulator of hypha-specific gene expression and it is thought to be the major regulator of hypha formation in response to different environmental exposures, like CO₂, pH and GlcNAc (Sudbery, 2011). *Efg1* mutant has abolished filamentation, cells show growth defects and a lack of filamentation in response to serum (Braun & Johnson, 2000). The second gene family originated in BCZ nodes encodes MEDA, which is a developmental modifier that spatially and temporally regulates the central transcription factor cascade in *Aspergillus fumigatus*. It is required for adherence, biofilm formation, conidiation, host cell interaction and virulence (Gravelat et al., 2010).

4.4.9. Vesicle transport

In „vesicle transport” category (Figure 24.) 107 genes (57 gene families) were collected, related to hyphal morphogenesis. A continuous reduction was observed in ancestral gene copy numbers along the backbone after the split of metazoa and fungi, and a remarkable difference was found between extant gene copy numbers of Dikarya and Mucoromycotina/early diverging fungi/non fungal species. Big proportion of the investigated gene families showed ancient eukaryotic origin (41 families out of the 57, 72%). Compared to other functional categories, the vesicle transport-related gene families did not show noteworthy expansion in BCZ nodes and none of the families originated there. Only 2 families are interesting to mention. Three duplications were found in a flippase encoding gene family. Flippase orthologs have phospholipid-translocating ATPase activity. They primarily localized to the hyphal tip, the plasma membrane and the Spitzenkörper. Phospholipid flippases DNFA and DNFB (*Aspergillus nidulans*) localize to the site of polarized growth and endocytosis and may be involved in secretion.

4.4.10. Phylogenetic age distribution of hypha morphogenesis genes.

A general pattern that emerges from the analyses of the different functional categories is that most of the gene families are conserved across fungi (Figure 25.) and their evolutionary origins predate that of hyphae (the origins of these gene families are mapped before the BCZ nodes). This conserved group contains 181 families, which is 50% of the total 362 investigated gene families. The big proportion of conserved families indicates that fungi have co-opted several conserved eukaryotic functionalities for supporting hyphal multicellularity (Figure 26., 27., Appendix 2.). It means that these ancient eukaryotic systems (e.g. actin cytoskeleton remodeling, vesicle transport system, signaling etc.) that evolved primarily to fulfill different functions in early eukaryotes, changed or were fine-tuned during fungal evolution to serve others, such as polarized hyphal growth in filamentous fungi. We also found that conserved gene families related to septation, polarity establishment and maintenance, cell cycle regulation, vesicle transport and microtubule-based transport systems are generally more diverse in animals, non-fungal eukaryotes and their ancestors than in fungal species, suggesting that despite the key roles of these families in hypha morphogenesis and multicellularity, they evolved primarily by gene loss in fungi during evolution.

Figure 25. The conservation of hypha morphogenesis genes across fungi. Dark and light blue indicates that the given gene family is present and absent in a species' genome, respectively.

Beside these evolutionary conserved gene families, a significant proportion of hypha morphogenesis families (164 families, 45.3%) emerged after the origin of hyphal MC. The origin of these families mapped after the BCZ nodes, indicating lineage- and species-specific genetic innovations. These genes and gene families also play important role in hypha morphogenesis, but in a lineage-specific manner. Only 17 families (4.7%) showed direct connection with the evolution of hyphal multicellularity. These families originated in BCZ nodes, at the putative origins of hyphal growth and were conserved thereafter (Table 1., Figure 26., 27., Appendix 2.) along the fungal phylogeny, providing potential candidates that shaped the evolution of hyphal multicellularity. These include two families of transcriptional regulators, six related to cell wall biogenesis, three to actin cytoskeleton regulation, three to polarity maintenance, two families involved in signaling and one involved in cell cycle regulation. These fungal-specific families are good candidates for being key contributors to the evolution of multicellular hyphae. Their functions are summarized in Table 1. and the detailed functional descriptions are found in the discussion of the belonging category. The reconstructed phylogenetic age distribution of hypha morphogenesis genes is shown on Figure 26, 27. and Appendix 2., where different color-codings show whether the gene family/functional category originated before, after or at the time of the emergence of multicellular hyphae.

Figure 26. Reconstructed phylogenetic age distribution of hypha morphogenesis genes across the 71 species phylogeny. Green colour represents gene families older than hypha morphogenesis, purple colour represents gene families whose emergence coincides with the evolution of hypha morphogenesis, yellow colour represent young, species and lineage specific gene families.

Figure 27. Phylogenetic age distribution of hypha morphogenesis genes. Schematic outline of terminal hyphal cell is shown with genes marked by dots and colored by phylogenetic age. Genes whose origin (based on their containing gene family) predates that of hyphal multicellularity (green) dominate the hyphal morphogenetic machinery, followed by genes that originated after hyphal MC (yellow) and genes whose origin approximately coincides with that of hyphae (purple). Data based on only *Aspergillus fumigatus* orthologs. See Appendix 2. for gene names and IDs.

Emergence of gene family	<i>A. fumigatus</i> ortholog	<i>S. cerevisiae</i> ortholog	Functional category and putative function
Node B MRCA of Dikarya, Mucoromycota, Zoopagomycota, Chytridiomycota, Blastocladiomycota	Afu7g03870	PAN1	actin cytoskeleton: endocytic adaptor that triggers hyphae-specific recruitment of the Arp2/3 complex to sites of endocytosis, for the recycling of excess membrane in the subapical region during hyphal growth
	CRH3	UTR2	cell wall biogenesis: chitin transglycosylase, localized to sites of polarized growth, functions in the transfer of chitin to beta (1-6) and beta (1-3) glucans
	GEL7	GAS1	cell wall biogenesis: beta (1-3) glucanotransferase, involved in cell wall remodeling during fungal germination or branching
	Afu6g04940	BNR1	polarity establishment: mediates actin cable assembly in filamentous fungi and has a role in diverse morphogenetic processes
	Afu4g04120	BEM1	polarity establishment: actin cytoskeleton reorganizing factor
	STUA	PHD1	transcriptional regulation: mediates yeast-filament transition in <i>S. cerevisiae</i> , developmental modifier in <i>A. fumigatus</i> , that spatially and temporally regulates the central transcription factor cascade
	MEDA	NA	transcriptional regulation
Node C MRCA of Dikarya, Mucoromycota, Zoopagomycota, Chytridiomycota	Afu6g07910	SLM1	actin cytoskeleton: effector of PtdIns(4,5)P2, essential for cell growth and actin cytoskeleton polarization
	Afu8g04520	SLA1	actin cytoskeleton: actin cytoskeleton-regulatory complex protein, localized to the actin patches that form the sites of endocytosis
	Afu4g06130	WHI2	cell cycle regulation: required for cell cycle regulation and stimulates filamentous growth
	Afu4g00620	DFG5	cell wall biogenesis: mannosidase, involved in bud formation and filamentous growth
	Afu8g02320	NA	cell wall biogenesis: ortholog of <i>N. crassa</i> cps1 polysaccharide synthase, functions in cell wall biosynthesis
	CHSD	NA	cell wall biogenesis: class VI chitin synthase, role in chitin biosynthesis
	RGSB	RAX1	polarity establishment: bipolar budding in <i>S. cerevisiae</i>
	Afu2g08800	SSY1	signaling: component of the SPS plasma membrane amino acid sensor system
Node Z MRCA of Dikarya, Mucoromycota, Zoopagomycota	RICA	NA	signaling: GDP/GTP exchange factor for G proteins, role in regulating fungal development
	KRE6	KRE6	cell wall biogenesis: role in beta (1-6) glucan biosynthesis

Table 1. List of the 17 gene families whose emergence coincides with the evolution of hyphal MC based on COMPARE analysis. NA indicates the lack of *Aspergillus* ortholog in *Saccharomyces*.

Given the low number of gene families specific to multicellular fungi (the number of those families that mapped to BCZ nodes), we were interested in whether evolutionary innovations by gene duplications show a peak in BCZ nodes. Fisher's exact tests were performed for comparing the number of gene duplications mapped to BCZ nodes in each functional category to the genome-wide number of duplications in BCZ nodes, using total number of duplications across the tree as a reference. As a result, ninety-three (25.7%) of the 362 hyphal morphogenesis-related gene families showed duplications in BCZ nodes. See the complete list of genes and gene families which show duplications in BCZ nodes per functional categories in Appendix 3. Enrichment analyses on the same dataset however, revealed no individual gene family with significantly increased number of duplications in BCZ nodes relative to the rest of the phylogenetic tree (Benjamini-Hochberg corrected $p < 0.05$, Fisher's exact test). The same enrichment analysis which was not performed on individual gene families but on their 10 functional groups (Table 2.) showed that duplications are significantly enriched ($p < 0.05$) in “cell wall biogenesis” and “transcriptional regulation” categories, suggesting that the diversification of gene families related to these categories could have played roles in the evolution of hyphae.

functional category	p value	FDR (correction for multiple testing)	significant based on p value	significant based on FDR
actin cytoskeleton	1	1	0	0
adhesion	0.007836078	0.026120259	1	1
cell cycle regulation	0.173000829	0.216251036	0	0
cell wall biogenesis	0.000349774	0.003364772	1	1
microtubular transport	0.105766484	0.151094977	0	0
polarity establishment	0.072435515	0.120725858	0	0
septation	0.067904547	0.120725858	0	0
signaling	0.257110837	0.285678708	0	0
transcriptional regulation	0.000672954	0.003364772	1	1
vesicle transport	0.013327299	0.033318248	1	1

Table 2. Results of the Fisher's exact test to compare the number of duplications mapped to BCZ nodes in each functional category to the genome-wide number of duplications in BCZ nodes, using total number of duplications across the tree as a reference. Blue color shows significantly less duplication in BCZ nodes compared to genome-wide ($p < 0.05$), orange color shows significantly more duplication in BCZ nodes compared to genome-wide ($p < 0.05$).

Other two functional groups, “adhesion” and “vesicle transport” categories, on the other hand showed significantly less duplication in BCZ nodes compared to the genome wide

duplication numbers ($p < 0.05$). The rest of the functional groups showed no such enrichment of gene duplications, indicating that the evolution of hyphal multicellularity did not generally coincide with a major burst of gene duplication in BCZ nodes, as some considerations of the evolution of multicellularity predicted (Knoll, 2011; Knoll & Hewitt, 2011).

4.4.11. Structural properties of hypha morphogenesis-related genes

We were also interested in whether changes in structural properties of hypha morphogenesis-related genes show a correlation with the evolution of hyphal multicellularity. To answer this, a two-tailed Welch's t-test with pooled variance estimation was performed on the 10 different functional groups and the belonging gene families. Significant differences ($p < 0.05$) were observed in gene, coding sequence (CDS) or intron lengths between unicellular and multicellular fungi in 7 out of 10 functional groups (exceptions are “cell cycle regulation”, “signaling” and “transcriptional regulation” categories) (Figure 28., Appendix 4.). Coding sequences of septation and polarity establishment and maintenance genes were significantly longer in multicellular than in unicellular fungi ($P = 0.0017$; $P = 0.00012$, Figure 28.). An opposite pattern was observed in introns, which were on average longer in unicellular fungi in actin cytoskeleton-, polarity establishment and maintenance, septation, and vesicle transport-related genes (Figure 28., Appendix 4.). On the other hand, no significant changes in gene structure were detected in cell wall biogenesis and transcriptional regulation-related genes. It is very interesting, because these two categories of hypha morphogenesis-related genes displayed significantly increased duplication numbers in early filamentous fungi. Duplicated copies of these genes can serve as potential targets for evolutionary changes. For example, gene copies can be the subjects of exon shuffling, or insertions/deletions of different DNA segments can also occur, resulting in structural changes of the gene, and also functional changes of the encoded protein. However, according to our results, it seems that structural changes (e.g. changes in CDS and intron length) of cell wall biogenesis and transcriptional regulation-related genes – that showed increased duplicability coinciding with the evolution of hyphae – did not contribute to the evolution of hyphal multicellularity.

Taken together, these comparative analyses on gene structures revealed several modifications to gene length in multicellular fungi in different functional categories,

which, although individually are small changes, could have contributed to the evolution of hyphae.

Figure 28. Statistical comparison of basic structural properties of genes related to hyphal MC. Box plots display differences in gene, coding sequence (CDS) and intron lengths of 4 unicellular (yellow) and 39 multicellular fungi (blue) in 10 functional categories. P-values derived from independent two-tailed Welch's t-tests are given for each comparison

4.5. Phagocytotic genes were exapted for hypha morphogenesis

Phagocytosis is a subtype of endocytosis where a large solid particle binds to the cell surface and it is engulfed by the cell. Generally, this process is regulated by signaling pathways, beginning with the perception of extracellular particles via transmembrane receptors. These signals are then transduced through intracellular signaling networks that reorganize the actin cytoskeleton and direct phagocytosis. Our set of hypha morphogenesis genes included several entries associated with this process in non-fungal eukaryotes. This is surprising given that phagocytosis is not known in fungal species, as their rigid cell wall forms a physical barrier to it. We therefore examined the evolutionary history and fate of these phagocytosis-related genes in multicellular fungi based on the phagocytotic machinery of *Dictyostelium discoideum* (Bozzaro et al., 2008; Cardelli, 2001; Rivero, 2008) (Appendix 5.) and other eukaryotes (Mao & Finnemann, 2015).

Based on sequence similarity, altogether 3966 genes (414 gene families) were identified related to phagocytosis. These protein families contain different molecules related to particle recognition (talin A and B, discoidin 1 and phagocytic receptor 1), actin cytoskeleton regulators (comitin, ponticulin, formin, profilin, SCAR, WASP, coronin), small G proteins and RAC GTP-ases, which are important regulators of the actin cytoskeleton polarization and remodeling during phagocytosis. The COMPARE analysis of these clusters revealed major gene family diversification in multicellular animals, as they had much more phagocytosis-related genes (avg: 893) than unicellular protists (avg: 497), unicellular fungi (avg: 315) and filamentous fungi (avg: 280). Yeast-like fungi on the other hand, showed a big reduction in copy numbers of phagocytosis-related genes, the average was only 177. Detailed analyses of highly important gene families revealed that filamentous fungi have retained phagocytotic gene families (Figure 29.). For example, members of the ARP2/3 complex are conserved in filamentous fungi. In general, the ARP2/3 complex is involved in actin filament assembly and actin cytoskeleton rearrangements, therefore it is necessary for lamellipodial protrusion, particle internalization and cell adhesion (May et al., 2000). In filamentous fungi, the ARP2/3 complex is responsible for recycling excess membrane in the subapical zone during hyphal growth. Investigation of this protein complex in *Candida albicans* revealed that there is a link between actin cytoskeleton stability and hypha-specific gene expression.

Figure 29. Evolutionary history of phagocytosis-related gene families. Several phagocytotic gene families retained in filamentous fungi (DOCK, ELMO, WASP). WAVE family retained only in early fungi (Blastocladiomycota and Chytridiomycota), WASH family is represented only in non-fungal eukaryotes. Bubble size is proportional to ancestral and extant gene copy number (except DOCK, ELMO, WASP, WAVE and WASH, where bubble size is not comparable to the main mapping). Copy numbers of filamentous fungi are labelled with yellow.

Disruption of the ARP2/3 complex prevented the yeast-hypha morphological switch and also membrane invagination during endocytosis (Epp et al., 2010). The activity of small GTPase RAC proteins is also required for actin cytoskeleton remodeling, modifying cell shape and therefore for phagocytosis. RAC proteins activate the SCAR/WAVE proteins, the main activators of the ARP2/3 complex. This Rac-dependent actin reorganization is further activated by guanine nucleotide exchange factors (GEFs), like DOCK and ELMO (Engulfment and cell motility) proteins (Brzostowski et al., 2009; Xu & Jin, 2019). ELMO1/2 family members are conserved across eukaryotes and are also found in all filamentous fungi, but are convergently lost in budding and fission yeasts, and in *Malassezia globosa*, all of which have reduced capacity for hyphal growth. The DOCK (dedicator of cytokinesis, *Saccharomyces cerevisiae* DCK1 (Hope et al., 2008)) protein family, which interacts with ELMO proteins, is also retained in fungal species. Of the

broader Wiskott-Aldrich syndrome family of proteins (WASPs) stimulates the actin filament branching activity of the ARP2/3 complex, and functions on the organization of the actin cytoskeleton during phagocytosis. WASP family proteins are conserved across eukaryotes and contain 6 subfamilies (WASP, WAVE, WASH, WHAMM, WAWH and WAML) (Kollmar et al., 2012). Separate analyses of these subfamilies revealed that the WASP family is conserved across fungi, the WAVE family is only represented in early diverging fungi and non-fungal eukaryotes, whereas the WASH family has been completely lost in fungal species (Figure 29.). These findings are also consistent with recent reports (Kollmar et al., 2012; Torruella et al., 2018). The investigation of gene family origin and evolution of these phagocytosis-related genes revealed that several phagocytotic genes are conserved across fungi, despite the loss of phagocytosis itself. These results highlight exaptation as another mechanism for the recruitment of genes for hyphal growth in multicellular fungi. Also, these findings suggest, that the ancient genetic toolkit responsible for phagocytosis in *Dictyostelium* and other eukaryotes possibly adapted during fungal evolution for serving other purposes in hyphal growth and multicellularity. Similarly to exaptation/co-option of various genetic toolkits playing role in the evolution of multicellularity in different lineages (Abedin & King, 2010), the exaptation of phagocytotic-toolkit for new functions in fungi maybe is one of the driving forces of the evolution of hyphal multicellularity.

4.6. Genome-wide screen finds novel gene families linked to hypha morphogenesis and hyphal multicellularity

Although, with the collection and analysis of hypha morphogenesis-related gene families we did our utmost to give a complete picture about the genetic background of filamentous growth, probably there are additional gene families related to hyphal morphogenesis, waiting for characterization. Therefore, we next asked if there are further gene families with a potential connection to hyphal multicellularity. We reasoned that gene families underlying hyphal multicellularity should originate or diversify in BCZ nodes and be conserved in descendent filamentous fungi. A systematic genome-wide search was performed for gene families fitting these criteria covering the investigated 71 genomes. This search yielded 414 families (ANOVA, $p < 0.05$, Appendix File 3.). Out of the 414 families, we found 114 which originated in BCZ nodes, while the others showed

increased duplication rates that exceeded the expectation derived from genome-wide figures of gene duplication. See the complete list of the 414 gene families in Appendix File 3. The conservation of these putative hyphal multicellularity-related gene families across fungi (54 multicellular and 4 unicellular) is shown on Figure 30. These families included several known morphogenetic families (e.g. *BGT3*, *RGSB* and *GEL2* of *Aspergillus fumigatus*, *BEM1* and *RAX1* of *Saccharomyces cerevisiae*), genes involved in actin cytoskeleton remodeling and cell wall biogenesis (*CHIB1* chitinase and *CHSB* chitin synthase gene in *Aspergillus fumigatus*), mating, pheromone response (*GPAA* of *Aspergillus fumigatus*), sporulation and transporters, among others (Appendix File 3.). Several of the identified families contain genes with reported growth defects in *Aspergillus fumigatus* or *Saccharomyces cerevisiae*, indicating that our genome-wide search recovered genes relevant for hyphal multicellularity. For example, RAX proteins are major regulators of cellular morphogenesis and are involved in bud site selection in budding yeasts (Chen et al., 2000; Gonia et al., 2013), polarized growth in *Schizosaccharomyces pombe* (Choi et al., 2006) and polarity establishment and maintenance in filamentous fungi (Borkovich et al., 2004). We further detected a fungal-specific cluster of tropomyosins (TPM1 and TPM2 in *Saccharomyces cerevisiae*), which originated in the MRCA of Blastocladiomycota and other fungi and is involved in polarized cell growth and the stabilization of actin microfilaments (Pruyne & Bretscher, 2000). Another family containing DIP1 (*Schizosaccharomyces pombe*) homologs (Afu6gl2370 in *Aspergillus fumigatus*) emerged in the node uniting Chytridiomycota with higher fungi and contains a single gene per species afterwards, except an expansion in WGD Mucoromycota (Corrochano et al., 2016) and losses in the Saccharomycotina lineage. In *Schizosaccharomyces pombe*, DIP1 activates the ARP2/3 complex without preexisting actin cables (Basu & Chang, 2011; Wagner et al., 2013) and thus regulates the actin cytoskeleton, cortical actin patch assembly and endocytosis through a mechanism that seems to be specific to multicellular filamentous fungi. Finally, among others, we detected a family containing *Saccharomyces cerevisiae* DPP1 homologs, which regulate morphogenetic transitions in dimorphic fungi through the synthesis of the fungal signaling molecule farnesol (Nickerson et al., 2006), which prompts us to speculate that it might have contributed to the elaboration of farnesol-based communication in fungi. Collectively, the origin of these gene families in BCZ nodes makes them candidate key contributors to the evolution of hyphal growth and hyphal multicellularity.

Because the emergence of hyphal multicellularity overlaps with that of other fungal traits, it is challenging to unequivocally separate signals conferred by the emergence of these traits from those by hyphae. It is conceivable that a portion of the 414 gene families were detected because of signals conferred by phylogenetically co-distributed traits, not necessarily hyphal multicellularity itself (see (Beaulieu & O'Meara, 2016) for a conceptually analogous problem in taxonomic diversification). One such trait could be the osmotrophy, the feeding mode by the absorption of soluble goods generated by extracellular enzyme complexes (Richards & Talbot, 2018), characteristic to fungal species. Among the identified 414 gene families, 20 families were detected that showed strong correlation with hyphal multicellularity and were annotated as various membrane transporters; such families could conceivably be related to osmotrophy. Further, there were 84 gene families that are functionally uncharacterized and thus it is impossible to speculate about their roles in hyphal multicellularity. These uncharacterized gene families indicate that there are plenty of fungal genes that evolved in concert with hyphal multicellularity and await functional characterization to understand their exact roles in hyphal growth.

Figure 30. Origin of 414 gene families potentially related to the evolution of hyphal MC, identified by ANOVA ($p < 0.05$). 114 families originated in BCZ nodes, including known morphogenesis-related proteins (e.g. BGT3, RGSB, GEL2 of *A. fumigatus*, RAX1, BEM1, TPM1 and DPP1 from *S. cerevisiae*, DIP1 from *S. pombe*) labelled as blue bars on the tree. Red numbers represent the number of gene families originated at the respective branches. Heatmap next to the tree shows the conservation of the identified 414 gene families across fungi. Purple color shows the presence of an ortholog gene in a particular gene family. White represents the absence of an ortholog gene in a particular gene family.

4.7. Yeasts retain genes required for hypha morphogenesis

Yeast-like fungi are secondarily simplified organisms with reduced ability to form hyphae, that spend most of their life cycle as unicellular organisms (Harris, 2011; Nagy et al., 2014, Nagy, 2017; Stajich et al., 2009). Our ancestral character state reconstruction performed on the 71 species (Figure 9.) implies that yeasts derived from filamentous ancestors, and thus they represent a classic example of the reduction in complexity (O'Malley et al., 2016). They were hypothesized to have lost the capability to form multicellular filaments, even though rudimentary forms of hyphal growth (termed “pseudohyphae”) exist in most yeast species. To investigate whether this morphological reduction was associated with genomic changes, we scrutinized the fate of hypha morphogenesis-related genes in five predominantly yeast-like lineages (Saccharomycotina, Taphrinomycotina, Ustilaginomycotina, Tremellomycotina and Pucciniomycotina) (Nagy et al., 2014).

Figure 31. Secondarily simplified yeast-like fungi retain genes for hyphal MC. Figure shows the percentages of lost genes in main morphogenesis-related categories. Percentages were calculated relative to ancestral copy numbers inferred in the node preceding the origin of 5 yeast-like clades (Saccharomycotina, Taphrinomycotina, Pucciniomycotina, Ustilaginomycotina and Tremellomycotina). Significance of the enrichment of gene losses in each category relative to genome-wide figures of gene loss were determined by Fisher’s exact test and is shown above bars.

Figure 32. Reconstruction of ancestral gene copy numbers of microtubule-based transport genes along the fungal phylogeny. Secondly simplified (yeast-like) clades are highlighted in yellow. Bubble size is proportional to reconstructed ancestral and extant gene copy numbers across 19 gene families. Copy number distribution of each gene family is shown right to the tree.

Because yeast genomes have undergone extreme streamlining during evolution, we evaluated gene loss among hypha morphogenesis genes in comparison to genome-wide figures of gene loss in yeasts. Yeast species generally have fewer hypha morphogenesis-related genes and reconstructions indicate more losses than duplications along branches of yeast ancestors (Figure 31.). However, when we corrected for genome-wide reductions in gene number, we found that comparatively fewer hypha morphogenesis-related genes (35-46%) were lost than genes with other functions (46-59%, Figure 31., Appendix 6.). Gene loss in yeast clades is significantly depleted ($p < 0.05$, Fisher's exact test) in most groups of hyphal morphogenesis-related genes compared to genome-wide expectations. The analysis recovered only six cases where significantly more hypha morphogenesis-related genes were lost than expected ($p < 0.05$, Fisher's exact test, Appendix 6.). These included proteins mostly related to adhesion and microtubule-based transport, suggesting that these functions are to some extent dispensable for yeast clades. Contractions or complete losses of gene families related to the microtubule-based transport system are

particularly interesting from the perspective of long-range vesicular and nuclear transport, which is a key contributor for polarized growth of multicellular hyphae. We found contractions in yeast-like species in gamma-tubulin complex-, kinesin- and dynein coding genes families and also complete losses of dynactins, dynactin linking proteins that mediate the interactions between motor complex components and the cytoskeleton (RO-2 in *Neurospora crassa*, (Vierula & Mais, 1997)) and also gamma-tubulin complex protein coding genes (GCPC in *Aspergillus nidulans*) (Figure 32.). Comparing different yeast lineages, budding and fission yeast show the most losses in morphogenesis-related genes, consistent with the strongest reduction of hyphal growth abilities in these clades. NADPH oxidases are thought to be potential contributors to multicellular development and differentiation via redox signaling (Blackstone, 2000; Lalucque & Silar, 2003).

Figure 33. Reconstruction of ancestral gene copy numbers of NADPH-oxidase genes. Numbers at the branches represent gene duplications (+) and losses (-) inferred by COMPARE. Bubble size is proportional to reconstructed ancestral gene copy number. Copy number distribution for each species is shown right to the tree.

In filamentous fungi, NADPH oxidases (NOX) are responsible to maintain the apical dominance in hyphae – the suppression of additional polarity axes near to the growing hyphal tip – by generating reactive oxygen species at the tip. Interestingly, several genes related to polarity establishment and maintenance (*RAC1*, *CDC42*, *BEM1*), also regulate NOX function and localization (Semighini & Harris, 2008). Therefore, we investigated the evolutionary fate of NADPH oxidase-coding gene families in the 71 genomes (Figure 33.), focusing on yeast species.

Losses of *NOX* genes were observed in all yeast clades, with complete loss of the family in the Saccharomycotina, Ustilaginomycotina lineages, in *Schizosaccharomyces pombe* and *Cryptococcus neoformans* (Figure 33.), as reported previously (Nguyen et al., 2017).

Altogether 54-65% of hypha morphogenesis-related genes were retained in yeast genomes (Appendix 6.). For example, hardly any reductions were observed in PI4,5P2 binding proteins (SLM1, SLM2 in *Saccharomyces cerevisiae* (Fadri et al., 2005)), which are involved in the regulation of actin cytoskeleton organization and endosomal transport, or in class I myosins (MYOA in *Aspergillus nidulans* (McGoldrick et al., 1995), MYO5 in *Saccharomyces cerevisiae* (Goodson et al., 1996)), which are localized to the site of polarized cell growth and involved in secretion and also in cell wall biogenesis.

Collectively, these analyses suggest that hyphal morphogenesis-related genes are in general less dispensable for yeasts than are genes with other functions. This is consistent with most yeast-like fungi being able to switch to hyphal or pseudohyphal growth under certain conditions, but surprising given the widely held belief that yeasts have lost multicellularity during evolution (O'Malley et al., 2016). The fact that hyphal morphogenesis genes are not statistically significantly enriched among losses compared to genome-wide expectation, however, is not in conflict with the reduction of multicellular abilities in yeast-like fungi. The inferred gene losses, nevertheless, do indicate reductions in hyphal growth, but this reduction is smaller compared to other functions in the genome. This, in turn suggests, that the ability for multicellular growth is among the functions preferentially retained by yeast-like fungi.

5. Discussion

Evolving multicellularity was one of the greatest achievements of life. In this study our aim was to investigate the evolution of multicellularity, focusing mainly on hyphal multicellularity, which is a unique multicellular organization of fungi with a poorly investigated evolutionary origin. Performing a comparative phylogenomic analysis on 71 species, covering the most important representatives of fungi, animals and their unicellular relatives, we expanded our knowledge on the evolution of hyphal multicellularity, and revealed the genetic bases of this transition.

In contrast to animals, where the cell-to-cell communication and adhesion are thought to be key components for the evolution of increased complexity, we could not confirm their importance in hyphal multicellularity. We investigated gene family origin and evolution of main metazoan multicellularity-related gene families (e.g. receptors, kinases and adhesion-related proteins) across our 71-species phylogeny and we did not observe substantial expansion in Histidine and Serine/Threonine kinase, GPCR and adhesive protein repertoires in hyphal multicellular fungal species, that otherwise often correlates with multicellularity and complexity in metazoans (de Mendoza et al., 2014; Lim & Pawson, 2010; Suga et al., 2012). These results highlight important differences between fungal and animal multicellularity and show that the diversification of ‘classic’ metazoan multicellularity-related gene families can’t explain the multicellularity in hypha-forming fungi.

Beside these well-known multicellularity-related gene families we examined further gene families whose origin and evolutionary dynamics maybe better correlate with the evolution of hyphal multicellularity. To understand the genetic background of the evolution of hyphae, we reconstructed the evolutionary history of 362 known hypha morphogenesis-related gene families, by mapping gene duplications and losses onto our 71-species phylogeny. The investigation of gene family evolution mainly focused on gene duplication events at the origin of hyphal growth, which was previously localized to three nodes around the split of early fungal lineages, such as Blastocladiomycota, Chytridiomycota and Zoopagomycota.

The evolutionary origin and duplication/loss history of hyphal morphogenesis-related gene families showed a very mixed picture. We found that 50% (181) of the gene families were older than hypha morphogenesis itself. Significant proportion (71%, 128)

of these gene families was ancient and conserved across all eukaryotes with very few or no duplication events at the origin of hyphal multicellularity. Another subset of these “old” gene families comprises families that have deep eukaryotic origins, but also show elevated duplication numbers coincident with the evolution of hyphae. These gene families with elevated duplicability are mostly coming from two functional categories (e.g. “cell wall biogenesis and remodeling” and “transcriptional regulation”).

In the second category, there are gene families whose origin mapped to the origin of hyphal growth. Total 4.7 % (17) of gene families coincide with the evolution of hypha morphogenesis, representing *GEL1*, *PANI*, *BEM1* and *BNR1* containing families, among others. These gene families could have evolved *de novo* at the origin of hyphal growth representing evolutionary innovations related to the transition to hyphal multicellularity, or diverged so much that sequence homology is not detectable any more across species. There are potential candidates for both cases. For example, APSES protein families contain characteristic, fungal-specific InterPro domains. Due to this specificity, these APSES proteins possibly evolved in early fungi, coinciding with the origin of hyphal growth, and could represent fungal-specific innovations underlying the evolution of hyphal multicellularity. The formin and “regulator of G protein signaling” (RGS) families however contain proteins only occurring in fungal species (*BNR1* and *RAX1* from *Saccharomyces cerevisiae*, respectively), but their characteristic InterPro domains were not strictly fungi-specific, and also present in other eukaryotes. This domain occurrence could reflect common ancestry of these proteins in different phylogenetic groups, but their sequence homology is not detectable anymore, because of sequence divergence.

The third category represents newly evolved “young” gene families. These families originate after the emergence of hyphal growth and cover almost the other half (45.3% (164)) of the investigated gene families, and they are regarded as lineage and species-specific genetic innovations related to the evolution of hyphal growth.

Overall 25.7% (93) of the investigated gene families showed duplications at the origin of hyphae, and these gene duplication events were characteristic mainly in “cell wall biogenesis and remodeling” and “transcriptional regulation” categories. However, separate statistical analyses on morphogenesis-related gene families revealed no individual gene families having significantly elevated duplication rate at the origin of hyphal multicellularity. These observations indicate a lack of a major burst of gene family origin coincident with the evolution of hyphae, and also limited novelty by gene duplication events in regard to hyphal multicellularity.

We not only investigated the known hypha morphogenesis-related gene families, but also performed a genome-wide search for other hyphal multicellularity-related gene families and predicted a link to hyphal growth for another 414 families, 114 of which originated at the emergence of hyphal multicellularity. This search resulted in already known morphogenetic families but also uncharacterized ones with no information about their functions and roles in hyphal growth. These unknown gene families can serve as potential candidates for further functional studies and characterization, revealing additional details about the evolution of hyphal growth.

Additionally, we were curious about the evolutionary fate of hypha morphogenesis-related gene families in secondarily simplified yeast-like fungi that show reduced complexity, and spend their life mostly as unicells. The analysis of gene losses in five yeast-like lineages revealed that 54-65% of hypha morphogenesis-related genes were retained in yeasts species, suggesting that the genetic toolkit responsible for hyphal growth is less dispensable in yeasts than others. The finding that yeast species possess a relatively big proportion of the genetic toolkit responsible for hyphal growth, can be a possible explanation for why yeast-like species are able to produce elongated filaments, pseudohyphae or sometimes true hyphae in response to different kinds of external stimuli (e.g. presence of serum, deprivation of nutrients etc.).

Apart from *de novo* gene family birth and gene family divergence, other mechanisms can be important driving forces of evolutionary innovations too. For example, changes in amino acid sequences and protein domain compositions can also play role in the evolution of hyphal multicellularity. It is possible that structural changes of hyphal morphogenesis-related genes and proteins in multicellular fungi correlate with the emergence of hyphal multicellularity (Lozada-Chávez et al., 2018). There are examples of sequence-level changes or domain shuffling in relation to the evolution of hyphal growth. For example, the investigation of motor proteins in *Neurospora crassa* revealed that fungal kinesins are faster and much more processive than other conventional eukaryotic kinesins (Lakämper et al., 2003; Steinberg & Schliwa, 1995, 1996). This increased processivity is probably the result of selection for a more efficient long-range vesicle transport along the hyphal axis, which is indispensable for polarized growth. Similarly, class V and class VII chitin synthase genes gained an extra myosin motor domain in early fungi, providing higher efficiency in polarized chitin biosynthesis and deposition, and therefore also being a key contributor to hyphal growth (Fujiwara et al., 1997; Sebé-Pedrós et al., 2014; Takeshita et al., 2006). To investigate whether there are

additional examples of these kinds of structural changes contributing to hyphal growth and multicellularity, we investigated changes in gene architecture (e.g. intron and coding sequence length) of hyphal multicellularity-related genes. Our comparative analyses on gene structures revealed significant differences between hypha morphogenesis-related genes of unicellular and multicellular fungi, and showed that several modifications of CDS and intron length in multicellular fungi, although individually are small changes, but they could have contributed to the evolution of hyphal multicellularity. The aforementioned examples highlight the diversity of mechanisms that could contribute to the emergence of fungal hyphae, such as *de novo* gene family birth, gene family diversification, or changes in gene architectures.

In summary, the extensive co-option and exaptation of ancient eukaryotic genetic toolkits may have been the most important driving forces of the evolution of hyphal multicellularity, followed by gene duplication events at the origin of hyphal growth, and changes in gene structures, accompanied by only limited *de novo* gene family birth. These novel findings related to hyphal multicellularity in fungi mirror patterns of the evolution of other multicellular organisms such as animals and plants (e.g. refs Prochnik et al., 2010; Richter et al., 2018; Seb-Pedrs et al., 2017; Suga et al., 2013; Suga & Ruiz-Trillo, 2013) that gave rise to the hypothesis that in terms of genetic novelty, transitions to multicellular life represent a minor rather a major evolutionary step (Grosberg & Strathmann, 2007). Discoveries of the last few years revealed, that many multicellularity-related genes required for multicellular development are deeply conserved, present in both unicellular and multicellular organisms, also likely existed in their ancestral single celled precursors (Grau-Bove et al., 2017; King et al., 2008; Richter et al., 2018). The co-option/exaptation of these evolutionary conserved genetic toolkits in different lineages (e.g. the evolution of ECM in Volvox by the co-option of HRGP cell wall components (Abedin & King, 2010), or the co-option of cadherin and integrin functions for establishing multicellularity in metazoans (Abedin & King, 2008, 2010)) seem to be contributed to the evolution of multicellular development. Similarly, the co-option of several ancient eukaryotic functions related to actin cytoskeleton regulation, septum formation, vesicle transport, or the exaptation of phagocytotic-toolkit for novel functions fulfilling key roles in hyphal morphogenesis can be serve as potential driving forces to the evolution of hyphal multicellularity. The idea of major phenotypic transition with little genetic innovations (Richter et al., 2018; Seb-Pedrs et al., 2016) finds support in our observations made here on fungi. Our findings highlight how small changes in genes

and genomes may lead to key evolutionary novelties, such as multicellular hyphae. This 'tinkering' process (Jacob, 1977) is consistent with the gradual evolution of multicellular hyphae, and could explain why rudimentary forms of hyphae exist in early-diverging fungal lineages, such as Blastocladiomycota and Chytridiomycota (Dee et al., 2015).

In metazoans, the dynamic cytoskeletal network and endomembrane system are supposed to be key contributors to the evolution of complexity (Knoll, 2011). We can assume that the co-option of these cellular functions for hypha morphogenesis was key to the evolution of hyphal multicellularity as well, along with newly evolved gene families and species and lineage specific genetic innovations. Gene losses, exaptations and other evolutionary innovations, like the osmotrophic lifestyle or the chitinous fungal cell wall, on the other hand, might have locked early fungi into certain evolutionary routes, eliminating the possibility to retain or evolve alternative phenotypes (e.g. phagocytotic ability). This might have eventually established hyphal growth, as an optimal solution for fungi and opened the door for establishing novel and diverse differentiation programs, which might have been prerequisites to the repeated emergence of complex multicellular structures (e.g. sexual and asexual fruiting body-types, sclerotia) later in evolution (Nagy et al., 2018).

The evolution of multicellular hyphae shows several unique patterns compared to other multicellular structures. In contrast to colonial and aggregative multicellular organisms where the diversification of adhesion and signal transduction-related genes (e.g. Ser/Thr kinases, Histidine kinases, GPCRs) was crucial for the evolution of multicellularity (Grau-Bove et al., 2017; Niklas, 2014; Seb-Pedrs et al., 2013, 2017), we did not find supporting evidence for this in hyphal multicellularity. This finding can be explained by the unique growth mode of multicellular hyphae, which is different from metazoans and plants, and shares similarities with only the Oomycota lineage. While the canonical cell-to-cell communication and adhesion-related genes might not be key contributors to the development of hyphal multicellularity, there are evidences for active communication between neighboring hyphae (Cottier & Mhlschlegel, 2012; Fischer et al., 2018). In contrast to metazoans, where the communication is linked to cell surface receptors (see e.g. ref Kulkarni et al., 2005), the communication in hyphae relies on other mechanisms, like quorum sensing (Albuquerque & Casadevall, 2012; Cottier & Mhlschlegel, 2012; Nickerson et al., 2006) or mechanisms that are not known yet. Our observations suggest that the prerequisites for the development of hyphal multicellularity differs considerably from that of other lineages. These differences raise the possibility

that in addition to the aggregative and clonal modes of evolving multicellularity, hyphal multicellularity should be considered a third, qualitatively different way.

In conclusion, multicellular hyphae represent an extremely successful adaptation to terrestrial life, and cutting edge technologies like comparative phylogenomics opens the door to study the genetic underpinnings of its origin and evolution, and for further investigations on whether such major phenotypic transitions represent - in terms of genetic novelty - a major or a minor transition.

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List of References

- Abedin, M., & King, N. (2008). The premetazoan ancestry of cadherins. *Science*, 319(5865), 946–948. <https://doi.org/10.1126/science.1151084>
- Abedin, M., & King, N. (2010). Diverse evolutionary paths to cell adhesion. [NIH Public Access]. In *Trends in cell biology* (Vol. 20, Issue 12). <https://doi.org/10.1016/j.tcb.2010.08.002>
- Ahrendt, S. R., Quandt, C. A., Ciobanu, D., Clum, A., Salamov, A., Andreopoulos, B., Cheng, J.-F., Woyke, T., Pelin, A., Henrissat, B., Reynolds, N. K., Benny, G. L., Smith, M. E., James, T. Y., & Grigoriev, I. V. (2018). Leveraging single-cell genomics to expand the fungal tree of life [Nature Publishing Group]. In *Nature Microbiology* (Vol. 3, Issue 12). <https://doi.org/10.1038/s41564-018-0261-0>
- Albuquerque, P., & Casadevall, A. (2012). Quorum sensing in fungi--a review. *Medical Mycology*, 50(4), 337–345. <https://doi.org/10.3109/13693786.2011.652201>
- Alex, L. A., Korch, C., Selitrennikoff, C. P., & Simon, M. I. (1998). COS1, a two-component histidine kinase that is involved in hyphal development in the opportunistic pathogen *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America*, 95(12), 7069–7073. <https://doi.org/10.1073/pnas.95.12.7069>
- Alkahyyat, F., Ni, M., Kim, S. C., & Yu, J. H. (2015). The WOPR domain protein OsaA orchestrates development in *Aspergillus nidulans*. In *PLoS ONE* (Vol. 10, Issue 9). <https://doi.org/10.1371/journal.pone.0137554>
- Annino, T., Chen, Z. Q., Shulenin, S., Costantino, J., Thomas, L., Lou, H., Stefanov, S., & Dean, M. (2006). Evolution of the vertebrate ABC gene family: Analysis of gene birth and death. In *Genomics* (Vol. 88, Issue 1). <https://doi.org/10.1016/j.ygeno.2006.03.001>
- Araujo-Bazán, L., Peñalva, M. A., & Espeso, E. A. (2008). Preferential localization of the endocytic internalization machinery to hyphal tips underlies polarization of the actin cytoskeleton in *Aspergillus nidulans*. *Molecular Microbiology*, 67(4), 891–905. <https://doi.org/10.1111/j.1365-2958.2007.06102.x>
- Arkowitz, R. A., & Bassilana, M. (2011). Polarized growth in fungi: Symmetry breaking and hyphal formation [Elsevier Ltd]. In *Seminars in Cell and Developmental Biology* (Vol. 22, Issue 8). <https://doi.org/10.1016/j.semcdb.2011.08.010>
- Arroyo, J., Farkaš, V., Sanz, A. B., & Cabib, E. (2016). ‘Strengthening the fungal cell wall through chitin-glucan cross-links: effects on morphogenesis and cell integrity.’ *Cellular Microbiology*, 18(9), 1239–1250. <https://doi.org/10.1111/cmi.12615>
- Aufauvre-Brown, A., Mellado, E., Gow, N. A. R., & Holden, D. W. (1997). *Aspergillus fumigatus* chsE: A Gene Related to CHS3 of *Saccharomyces cerevisiae* and Important for Hyphal Growth and Conidiophore Development but Not Pathogenicity. *Fungal Genetics and Biology*, 21(1), 141–152. <https://doi.org/10.1006/FGBI.1997.0959>
- Bahn, Y.-S., Xue, C., Idnurm, A., Rutherford, J. C., Heitman, J., & Cardenas, M. E. (2007). Sensing the environment: lessons from fungi. *Nature Reviews Microbiology*, 5(1), 57–69. <https://doi.org/10.1038/nrmicro1578>
- Bar-Yosef, H., Gildor, T., Ramírez-Zavala, B., Schmauch, C., Weissman, Z., Pinsky, M., Naddaf, R., Morschhäuser, J., Arkowitz, R. A., & Kornitzer, D. (2018). A global analysis of kinase function in *Candida albicans* hyphal morphogenesis reveals a role for the endocytosis regulator *akl1*. In *Frontiers in Cellular and Infection Microbiology* (Vol. 8, Issue FEB). <https://doi.org/10.3389/fcimb.2018.00017>
- Basu, R., & Chang, F. (2011). Characterization of Dip1p Reveals a Switch in Arp2/3-Dependent Actin

- Assembly for Fission Yeast Endocytosis [Cell Press]. In *Current Biology* (Vol. 21, Issue 11). <https://doi.org/10.1016/J.CUB.2011.04.047>
- Baumann, S., Ko'nig, J., Koepke, J., & Feldbru'gge, M. (2014). Endosomal transport of septin mRNA and protein indicates local translation on endosomes and is required for correct septin filamentation. *EMBO Reports*, 15(1), 94–102. <https://doi.org/10.1002/embr.201338037>
- Beaulieu, J. M., & O'Meara, B. C. (2016). Detecting Hidden Diversification Shifts in Models of Trait-Dependent Speciation and Extinction. *Systematic Biology*, 65(4), 583–601. <https://doi.org/10.1093/sysbio/syw022>
- Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. In *Frontiers in Plant Science* (Vol. 10, Issue September). <https://doi.org/10.3389/fpls.2019.01068>
- Bengtson, S., Rasmussen, B., Ivarsson, M., Muhling, J., Broman, C., Marone, F., Stampanoni, M., & Bekker, A. (2017). Fungus-like mycelial fossils in 2.4-billion-year-old vesicular basalt [Macmillan Publishers Limited, part of Springer Nature.]. In *Nature Ecology and Evolution* (Vol. 1, Issue 6). <https://doi.org/10.1038/s41559-017-0141>
- Bensen, E. S., Clemente-Blanco, A., Finley, K. R., Correa-Bordes, J., & Berman, J. (2005). The Mitotic Cyclins Clb2p and Clb4p Affect Morphogenesis in *Candida albicans*. *Molecular Biology of the Cell*, 16(7), 3387–3400. <https://doi.org/10.1091/mbc.e04-12-1081>
- Berbee, M. L., James, T. Y., & Strullu-Derrien, C. (2017). Early Diverging Fungi: Diversity and Impact at the Dawn of Terrestrial Life. In *Annual Review of Microbiology* (Vol. 71, Issue 1). <https://doi.org/10.1146/annurev-micro-030117-020324>
- Berbee, M. L., & Taylor, J. W. (2010). Dating the molecular clock in fungi – how close are we? *Fungal Biology Reviews*, 24(1–2), 1–16. <https://doi.org/10.1016/j.fbr.2010.03.001>
- Bergen, L. G., Upshall, A., & Morris, N. R. (1984). S-phase, G2, and nuclear division mutants of *Aspergillus nidulans*. *Journal of Bacteriology*, 159(1), 114–119. <https://doi.org/10.1128/jb.159.1.114-119.1984>
- Berleman, J. E., & Kirby, J. R. (2007). Multicellular development in *Myxococcus xanthus* is stimulated by predator-prey interactions. In *Journal of Bacteriology* (Vol. 189, Issue 15). <https://doi.org/10.1128/JB.00544-07>
- Biswas, S., Van Dijck, P., & Datta, A. (2007). Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of *Candida albicans*. *Microbiology and Molecular Biology Reviews : MMBR*, 71(2), 348–376. <https://doi.org/10.1128/MMBR.00009-06>
- Blackstone, N. W. (2000). Redox control and the evolution of multicellularity. In *BioEssays* (Vol. 22, Issue 10). [https://doi.org/10.1002/1521-1878\(200010\)22:10<947::AID-BIES10>3.0.CO;2-W](https://doi.org/10.1002/1521-1878(200010)22:10<947::AID-BIES10>3.0.CO;2-W)
- Bonner, J. T. (1965). *Size and cycle*. Princeton, NJ: Princeton University Press.
- Bonner, J. T. (2004). Perspective: The size-complexity rule. In *Evolution* (Vol. 58, Issue 9). <https://doi.org/10.1111/j.0014-3820.2004.tb00476.x>
- Bonner, J. T. (1998). The origins of multicellularity. In *Integrative Biology: Issues, News, and Reviews* (Vol. 1, Issue 1). [https://doi.org/10.1002/\(sici\)1520-6602\(1998\)1:1<27::aid-inbi4>3.3.co;2-y](https://doi.org/10.1002/(sici)1520-6602(1998)1:1<27::aid-inbi4>3.3.co;2-y)
- Bonner, J. T. (2009). First signals: The evolution of multicellular development. In *First Signals: The Evolution of Multicellular Development*. <https://doi.org/10.5860/choice.38-6161>
- Bonneville, S., Delpomdor, F., Preat, A., Chevalier, C., Araki, T., Kazemian, M., Steele, A., Schreiber, A., Wirth, R., & Benning, L. G. (2020). Molecular identification of fungi microfossils in a

- Neoproterozoic shale rock. In *Science Advances* (Vol. 6, Issue 4).
<https://doi.org/10.1126/sciadv.aax7599>
- Boraas, M. E., Seale, D. B., & Boxhorn, J. E. (1998). Phagotrophy by flagellate selects for colonial prey: A possible origin of multicellularity. In *Evolutionary Ecology* (Vol. 12, Issue 2).
<https://doi.org/10.1023/A:1006527528063>
- Borkovich, K. A., Alex, L. A., Yarden, O., Freitag, M., Turner, G. E., Read, N. D., Seiler, S., Bell-Pedersen, D., Paietta, J., Plesofsky, N., Plamann, M., Goodrich-Tanrikulu, M., Schulte, U., Mannhaupt, G., Nargang, F. E., Radford, A., Selitrennikoff, C., Galagan, J. E., Dunlap, J. C., ... Pratt, R. (2004). Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. *Microbiology and Molecular Biology Reviews* : *MMBR*, 68(1), 1–108. <https://doi.org/10.1128/MMBR.68.1.1-108.2004>
- Bozzaro, S., Bucci, C., & Steinert, M. (2008). Chapter 6 Phagocytosis and Host–Pathogen Interactions in Dictyostelium with a Look at Macrophages. In *International review of cell and molecular biology* (Vol. 271). [https://doi.org/10.1016/S1937-6448\(08\)01206-9](https://doi.org/10.1016/S1937-6448(08)01206-9)
- Brand, A. (2012). Hyphal growth in human fungal pathogens and its role in virulence. In *International Journal of Microbiology* (Vol. 2012). <https://doi.org/10.1155/2012/517529>
- Brand, A., & Gow, N. A. (2009). Mechanisms of hypha orientation of fungi [Elsevier Ltd]. In *Current Opinion in Microbiology* (Vol. 12, Issue 4). <https://doi.org/10.1016/j.mib.2009.05.007>
- Branda, S. S., González-Pastor, J. E., Ben-Yehuda, S., Losick, R., & Kolter, R. (2001). Fruiting body formation by *Bacillus subtilis*. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 98, Issue 20). <https://doi.org/10.1073/pnas.191384198>
- Brasier, C. M. (1991). *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. In *Mycopathologia* (Vol. 115, Issue 3). <https://doi.org/10.1007/BF00462219>
- Braun, B. R., & Johnson, A. D. (2000). TUP1, CPH1 and EFG1 make independent contributions to filamentation in *Candida albicans*. In *Genetics* (Vol. 155, Issue 1).
- Brefort, T., Doehlemann, G., Mendoza-Mendoza, A., Reissmann, S., Djamei, A., & Kahmann, R. (2009). *Ustilago maydis* as a Pathogen . In *Annual Review of Phytopathology* (Vol. 47, Issue 1).
<https://doi.org/10.1146/annurev-phyto-080508-081923>
- Brewster, J. L., de Valoir, T., Dwyer, N. D., Winter, E., & Gustin, M. C. (1993). An osmosensing signal transduction pathway in yeast. *Science*, 259(5102), 1760 LP – 1763.
<https://doi.org/10.1126/science.7681220>
- Bridges, C. B. 1936. The *Bar* „gene” a duplication. *Science* 83(2148):210-211
- Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden killers: Human fungal infections. In *Science Translational Medicine* (Vol. 4, Issue 165).
<https://doi.org/10.1126/scitranslmed.3004404>
- Brown, M. W., Spiegel, F. W., & Silberman, J. D. (2009). Phylogeny of the “forgotten” cellular slime mold, *Fonticula alba*, reveals a key evolutionary branch within Opisthokonta. In *Molecular Biology and Evolution* (Vol. 26, Issue 12). <https://doi.org/10.1093/molbev/msp185>
- Brundrett, M. C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. In *Plant and Soil* (Vol. 320, Issues 1–2).
<https://doi.org/10.1007/s11104-008-9877-9>
- Brunet, T., & King, N. (2017). The Origin of Animal Multicellularity and Cell Differentiation. In *Dev Cell* (Vol. 43, Issue 2). <https://doi.org/10.1016/j.devcel.2017.09.016>

- Brzostowski, J. A., Fey, P., Yan, J., Isik, N., & Jin, T. (2009). The Elmo family forms an ancient group of actin-regulating proteins. In *Communicative and Integrative Biology* (Vol. 2, Issue 4). <https://doi.org/10.4161/cib.2.4.8549>
- Buss, L. W. (1987). *The evolution of individuality*. Princeton, NJ: Princeton University Press.
- Butterfield, N. J. (2005). Probable Proterozoic fungi. *Paleobiology*, 31(1), 165–182. [https://doi.org/10.1666/0094-8373\(2005\)031<0165:ppf>2.0.co;2](https://doi.org/10.1666/0094-8373(2005)031<0165:ppf>2.0.co;2)
- Cain, C. W., Lohse, M. B., Homann, O. R., Sil, A., & Johnson, A. D. (2012). A conserved transcriptional regulator governs fungal morphology in widely diverged species. [Genetics]. In *Genetics* (Vol. 190, Issue 2). <https://doi.org/10.1534/genetics.111.134080>
- Callejas-Negrete, O. A., Plamann, M., Schnittker, R., Bartnicki-García, S., Roberson, R. W., Pimienta, G., & Mouriño-Pérez, R. R. (2015). Two microtubule-plus-end binding proteins LIS1-1 and LIS1-2, homologues of human LIS1 in *Neurospora crassa* [Academic Press]. In *Fungal Genetics and Biology* (Vol. 82). <https://doi.org/10.1016/J.FGB.2015.07.009>
- Capella-Gutierrez, S., Silla-Martinez, J. M., & Gabaldon, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25(15), 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Cardelli, J. (2001). Phagocytosis and macropinocytosis in *Dictyostelium*: Phosphoinositide-based processes, biochemically distinct. In *Traffic* (Vol. 2, Issue 5). <https://doi.org/10.1034/j.1600-0854.2001.002005311.x>
- Carroll, S. B. (2001). Chance and necessity: The evolution of morphological complexity and diversity. *Nature*, 409, 1102–1109.
- Cereijido, M., Contreras, R. G., & Shoshani, L. (2004). Cell adhesion, polarity, and epithelia in the dawn of metazoans. In *Physiological Reviews* (Vol. 84, Issue 4). <https://doi.org/10.1152/physrev.00001.2004>
- Chang, Y., Wang, S., Sekimoto, S., Aerts, A. L., Choi, C., Clum, A., LaButti, K. M., Lindquist, E. A., Yee Ngan, C., Ohm, R. A., Salamov, A. A., Grigoriev, I. V., Spatafora, J. W., & Berbee, M. L. (2015). Phylogenomic Analyses Indicate that Early Fungi Evolved Digesting Cell Walls of Algal Ancestors of Land Plants. *Genome Biology and Evolution*, 7(6), 1590–1601. <https://doi.org/10.1093/gbe/evv090>
- Chen, K., Durand, D., & Farach-Colton, M. (2000). NOTUNG: A Program for Dating Gene Duplications and Optimizing Gene Family Trees. *Journal of Computational Biology*, 7(3–4), 429–447. <https://doi.org/10.1089/106652700750050871>
- Chen, T., Hiroko, T., Chaudhuri, A., Inose, F., Lord, M., Tanaka, S., Chant, J., & Fujita, A. (2000). Multigenerational Cortical Inheritance of the Rax2 Protein in Orienting Polarity and Division in Yeast. *Science*, 290(5498), 1975–1978. <https://doi.org/10.1126/SCIENCE.290.5498.1975>
- Chenevert, J., Corrado, K., Bender, A., Pringle, J., & Herskowitz, I. (1992). A yeast gene (BEM1) necessary for cell polarization whose product contains two SH3 domains. *Nature*, 356(6364), 77–79. <https://doi.org/10.1038/356077a0>
- Cheon, S. A., Bal, J., Song, Y., Hwang, H., Kim, A. R., Kang, W. K., Kang, H. A., Hannibal-Bach, H. K., Knudsen, J., Ejlsing, C. S., & Kim, J.-Y. (2012). Distinct roles of two ceramide synthases, CaLag1p and CaLac1p, in the morphogenesis of *Candida albicans*. *Molecular Microbiology*, 83(4), 728–745. <https://doi.org/10.1111/j.1365-2958.2011.07961.x>
- Choi, E., Lee, K., & Song, K. (2006). Function of rax2p in the polarized growth of fission yeast. In *Molecules and cells* (Vol. 22, Issue 2). <http://www.ncbi.nlm.nih.gov/pubmed/17085965>
- Corrochano, L. M., Kuo, A., Marcet-Houben, M., Polaino, S., Salamov, A., Villalobos-Escobedo, J. M.,

- Grimwood, J., Álvarez, M. I., Avalos, J., Bauer, D., Benito, E. P., Benoit, I., Burger, G., Camino, L. P., Cánovas, D., Cerdá-Olmedo, E., Cheng, J.-F., Domínguez, A., Eliáš, M., ... Grigoriev, I. V. (2016). Expansion of Signal Transduction Pathways in Fungi by Extensive Genome Duplication. *Current Biology*, 26(12), 1577–1584. <https://doi.org/10.1016/J.CUB.2016.04.038>
- Cottier, F., & Mühlischlegel, F. A. (2012). Communication in Fungi. In *International Journal of Microbiology* (Vol. 2012). <https://doi.org/10.1155/2012/351832>
- Daher, F. B., & Braybrook, S. A. (2015). How to let go: Pectin and plant cell adhesion. In *Frontiers in Plant Science* (Vol. 6, Issue JULY). <https://doi.org/10.3389/fpls.2015.00523>
- Darling, A. E., Carey, L., & Feng, W. (2003). The Design , Implementation , and Evaluation of mpiBLAST. *Proceedings of ClusterWorld*, 2003, 13–15. <https://doi.org/10.1177/1740774509338234>
- de Mendoza, A., Sebé-Pedrós, A., & Ruiz-Trillo, I. (2014). The Evolution of the GPCR Signaling System in Eukaryotes: Modularity, Conservation, and the Transition to Metazoan Multicellularity. *Genome Biology and Evolution*, 6(3), 606–619. <https://doi.org/10.1093/gbe/evu038>
- Dee, J. M., Mollicone, M., Longcore, J. E., Roberson, R. W., & Berbee, M. L. (2015). Cytology and molecular phylogenetics of Monoblepharidomycetes provide evidence for multiple independent origins of the hyphal habit in the Fungi. *Mycologia*, 107(4), 710–728. <https://doi.org/10.3852/14-275>
- Defosse, T. A., Sharma, A., Mondal, A. K., Dugé de Bernonville, T., Latgé, J.-P., Calderone, R., Giglioli-Guivarc'h, N., Courdavault, V., Clastre, M., & Papon, N. (2015). Hybrid histidine kinases in pathogenic fungi. *Molecular Microbiology*, 95(6), 914–924. <https://doi.org/10.1111/mmi.12911>
- Dotzler, N., Krings, M., Taylor, T. N., & Agerer, R. (2006). Germination shields in Scutellospora (Glomeromycota: Diversisporales, Gigasporaceae) from the 400 million-year-old Rhynie chert. *Mycological Progress*, 5(3), 178–184. <https://doi.org/10.1007/s11557-006-0511-z>
- Dotzler, N., Walker, C., Krings, M., Hass, H., Kerp, H., Taylor, T. N., & Agerer, R. (2009). Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. *Mycological Progress*, 8(1), 9–18. <https://doi.org/10.1007/s11557-008-0573-1>
- Du, Q., Kawabe, Y., Schilde, C., Chen, Z., & Schaap, P. (2015). The Evolution of Aggregative Multicellularity and Cell–Cell Communication in the Dictyostelia [Elsevier]. In *Journal of Molecular Biology* (Vol. 427, Issue 23). <https://doi.org/10.1016/j.jmb.2015.08.008>
- Edgington, N. P., Blacketer, M. J., Bierwagen, T. A., Myers, A. M. (1998). Control of *Saccharomyces cerevisiae* Filamentous Growth by Cyclin-Dependent Kinase Cdc28. *Molecular And Cellular Biology*, p. 1369-1380. <https://doi.org/10.1128/MCB.19.2.1369>
- Epp, E., Nazarova, E., Regan, H., Douglas, L. M., Konopka, J. B., Vogel, J., & Whiteway, M. (2013). Clathrin- and Arp2/3-independent endocytosis in the fungal pathogen *Candida albicans*. *MBio*, 4(5), e00476-13. <https://doi.org/10.1128/mBio.00476-13>
- Epp, E., Walther, A., Lépine, G., Leon, Z., Mullick, A., Raymond, M., Wendland, J., & Whiteway, M. (2010). Forward genetics in *Candida albicans* that reveals the Arp2/3 complex is required for hyphal formation, but not endocytosis. In *Molecular Microbiology* (Vol. 75, Issue 5). <https://doi.org/10.1111/j.1365-2958.2009.07038.x>
- Evans, C., & Hedger, J. (2001). Degradation of plant cell wall polymers. In G. Gadd (Ed.), *Fungi in Bioremediation* (British Mycological Society Symposia, pp. 1-26). Cambridge: Cambridge University Press. doi:10.1017/CBO9780511541780.002
- Evans, C. S., & Hedger, J. N. (2009). Degradation of plant cell wall polymers. In *Fungi in Bioremediation*. <https://doi.org/10.1017/cbo9780511541780.002>

- Fadri, M., Daquinag, A., Wang, S., Xue, T., & Kunz, J. (2005). The Pleckstrin Homology Domain Proteins Slm1 and Slm2 Are Required for Actin Cytoskeleton Organization in Yeast and Bind Phosphatidylinositol-4,5-Bisphosphate and TORC2. *Molecular Biology of the Cell*, 16(4), 1883–1900. <https://doi.org/10.1091/mbc.e04-07-0564>
- Fairclough, S. R., Chen, Z., Kramer, E., Zeng, Q., Young, S., Robertson, H. M., Begovic, E., Richter, D. J., Russ, C., Westbrook, M. J., Manning, G., Lang, B. F., Haas, B., Nusbaum, C., & King, N. (2013). Premetazoan genome evolution and the regulation of cell differentiation in the choanoflagellate *Salpingoeca rosetta* [BioMed Central]. In *Genome Biology* (Vol. 14, Issue 2). <https://doi.org/10.1186/gb-2013-14-2-r15>
- Fairclough, S. R., Dayel, M. J., & King, N. (2010). Multicellular Development in a Choanoflagellate. *Current Biology : CB*, 20(20), R875–R876. <https://doi.org/10.1016/j.cub.2010.09.014>
- Felsenstein, J., Ackerly, D. D., & Mcpeck, M. A. (2012). A comparative method for both discrete and continuous characters using the threshold model. In *American Naturalist* (Vol. 179, Issue 2). <https://doi.org/10.1086/663681>
- Finkel, J. S., & Mitchell, A. P. (2011). Genetic control of *Candida albicans* biofilm development. *Nature Reviews Microbiology*, 9(2), 109–118. <https://doi.org/10.1038/nrmicro2475>
- Fischer, M. S., Wu, V. W., Lee, J. E., O'Malley, R. C., & Glass, N. L. (2018). Regulation of Cell-to-Cell Communication and Cell Wall Integrity by a Network of MAP Kinase Pathways and Transcription Factors in *Neurospora crassa*. [Genetics]. In *Genetics* (Vol. 209, Issue 2). <https://doi.org/10.1534/genetics.118.300904>
- Fischer, R., Zekert, N., & Takeshita, N. (2008). Polarized growth in fungi – interplay between the cytoskeleton, positional markers and membrane domains. *Molecular Microbiology*, 68(4), 813–826. <https://doi.org/10.1111/j.1365-2958.2008.06193.x>
- Fisher, K. E., & Roberson, R. W. (2016). Hyphal tip cytoplasmic organization in four zygomycetous fungi. In *Mycologia* (Vol. 108, Issue 3). <https://doi.org/10.3852/15-226>
- Fisher, K. E., Romberger, I., Lowry, D., Shange, P., & Roberson, R. W. (2018). Hyphal tip growth and cytoplasmic characters of *Conidiobolus coronatus* (Zoopagomycota, Entomophthoromycotina) [Taylor & Francis]. In *Mycologia* (Vol. 110, Issue 1). <https://doi.org/10.1080/00275514.2017.1403808>
- Force, A., Lynch, M., Pickett, F. B., Amores, A., Yan, Y. L., & Postlethwait, J. (1999). Preservation of duplicate genes by complementary, degenerative mutations. In *Genetics* (Vol. 151, Issue 4).
- Ford, S. K., & Pringle, J. R. (1991). Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: Localization of the CDC11 gene product and the timing of events at the budding site. *Developmental Genetics*, 12(4), 281–292. <https://doi.org/10.1002/dvg.1020120405>
- Fortwendel, J. R., Juvvadi, P. R., Rogg, L. E., Asfaw, Y. G., Burns, K. A., Randell, S. H., & Steinbach, W. J. (2012). Plasma Membrane Localization Is Required for RasA-Mediated Polarized Morphogenesis and Virulence of *Aspergillus fumigatus*. *Eukaryotic Cell*, 11(8), 966–977. <https://doi.org/10.1128/EC.00091-12>
- Fricker, M. D., Heaton, L. L. M., Jones, N. S., & Boddy, L. (2017). The Mycelium as a Network. In *Microbiology Spectrum* (Vol. 5, Issue 3). <https://doi.org/doi:10.1128/microbiolspec.FUNK-0033-2017>
- Fu, C., Sokolow, E., Rupert, C. B., & Free, S. J. (2014). The *Neurospora crassa* CPS-1 polysaccharide synthase functions in cell wall biosynthesis. *Fungal Genetics and Biology*, 69, 23–30. <https://doi.org/10.1016/J.FGB.2014.05.009>
- Fujita, A., Lord, M., Hiroko, T., Hiroko, F., Chen, T., Oka, C., Misumi, Y., & Chant, J. (2004). Rax1, a protein required for the establishment of the bipolar budding pattern in yeast [Elsevier]. In *Gene*

- (Vol. 327, Issue 2). <https://doi.org/10.1016/J.GENE.2003.11.021>
- Fujiwara, M., Horiuchi, H., Ohta, A., & Takagi, M. (1997). A Novel Fungal Gene Encoding Chitin Synthase with a Myosin Motor-like Domain. *Biochemical and Biophysical Research Communications*, 236(1), 75–78. <https://doi.org/10.1006/BBRC.1997.6907>
- Gauthier, G. M. (2015). Dimorphism in Fungal Pathogens of Mammals, Plants, and Insects. In *PLoS Pathogens* (Vol. 11, Issue 2). <https://doi.org/10.1371/journal.ppat.1004608>
- Gauthier, G. M. (2017). Fungal dimorphism and virulence: Molecular mechanisms for temperature adaptation, immune evasion, and in vivo survival. In *Mediators of Inflammation* (Vol. 2017). <https://doi.org/10.1155/2017/8491383>
- Gazave, E., Lapébie, P., Richards, G. S., Brunet, F., Ereskovsky, A. V., Degnan, B. M., Borchellini, C., Vervoort, M., & Renard, E. (2009). Origin and evolution of the Notch signalling pathway: An overview from eukaryotic genomes. In *BMC Evolutionary Biology* (Vol. 9, Issue 1). <https://doi.org/10.1186/1471-2148-9-249>
- Gil-Bona, A., Parra-Giraldo, C. M., Hernández, M. L., Reales-Calderon, J. A., Solis, N. V., Filler, S. G., & Monteoliva, L. (2015). *Candida albicans* cell shaving uncovers new proteins involved in cell wall integrity, yeast to hypha transition, stress response and host–pathogen interaction. *Journal of Proteomics*, 127, 340–351. <https://doi.org/10.1016/J.JPROT.2015.06.006>
- Gimeno, C. J., & Fink, G. R. (1994). Induction of pseudohyphal growth by overexpression of PHD1, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. *Molecular and Cellular Biology*, 14(3), 2100–2112. <https://doi.org/10.1128/MCB.14.3.2100>
- Glöckner, G., Lawal, H. M., Felder, M., Singh, R., Singer, G., Weijer, C. J., & Schaap, P. (2016). The multicellularity genes of dictyostelid social amoebas. *Nature Communications*, 7(1), 12085. <https://doi.org/10.1038/ncomms12085>
- Gonia, S., Norton, J., Watanaskul, L., Pulver, R., Morrison, E., Brand, A., & Gale, C. A. (2013). Rax2 is important for directional establishment of growth sites, but not for reorientation of growth axes, during *Candida albicans* hyphal morphogenesis. *Fungal Genetics and Biology: FG & B*, 56, 116–124. <https://doi.org/10.1016/j.fgb.2013.04.002>
- González-Novo, A., Correa-Bordes, J., Labrador, L., Sánchez, M., Vázquez de Aldana, C. R., & Jiménez, J. (2008). Sep7 Is Essential to Modify Septin Ring Dynamics and Inhibit Cell Separation during *Candida albicans* Hyphal Growth. *Molecular Biology of the Cell*, 19(4), 1509–1518. <https://doi.org/10.1091/mbc.e07-09-0876>
- Goodson, H. V., Anderson, B. L., Warrick, H. M., Pon, L. A., & Spudich, J. A. (1996). Synthetic lethality screen identifies a novel yeast myosin I gene (MYO5): myosin I proteins are required for polarization of the actin cytoskeleton. *The Journal of Cell Biology*, 133(6), 1277–1291. <https://doi.org/10.1083/JCB.133.6.1277>
- Gould, S. J., & Vrba, E. S. (1982). Exaptation—a Missing Term in the Science of Form. *Paleobiology*, 1(N/A), 4–15. <https://doi.org/10.1017/S0094837300004310>
- Grau-Bove, X., Torruella, G., Donachie, S., Suga, H., Leonard, G., Richards, T. A., & Ruiz-Trillo, I. (2017). Dynamics of genomic innovation in the unicellular ancestry of animals. *Elife*, 6. <https://doi.org/10.7554/eLife.26036>
- Graur, D. (2016). *Molecular and Genome Evolution*. Sunderland, MA, Sinauer Associates, Inc. Publishers
- Gravelat, F. N., Ejzykowicz, D. E., Chiang, L. Y., Chabot, J. C., Urb, M., Macdonald, K. D., al-Bader, N., Filler, S. G., & Sheppard, D. C. (2010). *Aspergillus fumigatus* MedA governs adherence, host cell interactions and virulence. *Cellular Microbiology*, 12(4), 473–488. <https://doi.org/10.1111/j.1462-5822.2009.01408.x>

- Grosberg, R. K., & Strathmann, R. R. (2007). The Evolution of Multicellularity: A Minor Major Transition? [Annual Reviews]. In *Annual Review of Ecology, Evolution, and Systematics* (Vol. 38, Issue 1). <https://doi.org/10.1146/annurev.ecolsys.36.102403.114735>
- Gull, K., & Trinci, A. P. (1975). Septal Ultrastructure In *Basidiobolus ranarum*. *Sabouraudia*, Volume 13, Issue 1, January 1975, Pages 49–51, <https://doi.org/10.1080/00362177585190101>
- Gupta, G. D., Free, S. J., Levina, N. N., Keränen, S., & Heath, I. B. (2003). Two divergent plasma membrane syntaxin-like SNAREs, nsyn1 and nsyn2, contribute to hyphal tip growth and other developmental processes in *Neurospora crassa* [Academic Press]. In *Fungal Genetics and Biology* (Vol. 40, Issue 3). [https://doi.org/10.1016/S1087-1845\(03\)00109-9](https://doi.org/10.1016/S1087-1845(03)00109-9)
- Gupta, G. D., & Heath, I. B. (2000). A Tip-High Gradient of a Putative Plasma Membrane SNARE Approximates the Exocytotic Gradient in Hyphal Apices of the Fungus *Neurospora crassa*. *Fungal Genetics and Biology*, 29(3), 187–199. <https://doi.org/10.1006/fgbi.2000.1200>
- Haldane J. B. S. (1932). *The Causes of Evolution*. Longmans, London
- Halder, G., & Johnson, R. L. (2011). Hippo signaling: growth control and beyond. *Development*, 138, 9-22. <https://doi.org/10.1242/dev.045500>
- Han, K.-H., & Prade, R. A. (2002). Osmotic stress-coupled maintenance of polar growth in *Aspergillus nidulans*. *Molecular Microbiology*, 43(5), 1065–1078. <https://doi.org/10.1046/j.1365-2958.2002.02774.x>
- Han, Q., Wang, N., Yao, G., Mu, C., Wang, Y., & Sang, J. (2019). Blocking β -1,6-glucan synthesis by deleting KRE6 and SKN1 attenuates the virulence of *Candida albicans*. In *Molecular Microbiology* (Vol. 111, Issue 3). <https://doi.org/10.1111/mmi.14176>
- Haneef, M., Ceseracciu, L., Canale, C., Bayer, I. S., Heredia-Guerrero, J. A., Athanassiou, A. (2017). Advanced Materials From Fungal Mycelium: Fabrication and Tuning of Physical Properties. *Scientific Reports*, 7:41292. <https://doi.org/10.1038/srep41292>
- Harris, S. D. (2008). Branching of fungal hyphae: Regulation, mechanisms and comparison with other branching systems. In *Mycologia* (Vol. 100, Issue 6). <https://doi.org/10.3852/08-177>
- Harris, S. D. (2011). Hyphal morphogenesis: an evolutionary perspective. In *Fungal Biology* (Vol. 115, Issue 6). <https://doi.org/10.1016/j.funbio.2011.02.002>
- Harris, S. D., Hamer, L., Sharpless, K. E., & Hamer, J. E. (1997). The *Aspergillus nidulans* sepA gene encodes an FH1/2 protein involved in cytokinesis and the maintenance of cellular polarity. In *EMBO Journal* (Vol. 16, Issue 12). <https://doi.org/10.1093/emboj/16.12.3474>
- Harris, S. D., Hofmann, A. F., Tedford, H. W., & Lee, M. P. (1999). Identification and Characterization of Genes Required for Hyphal Morphogenesis in the Filamentous Fungus *Aspergillus nidulans*. *Genetics*, 151(3).
- Harris, S. D., & Momany, M. (2004). Polarity in filamentous fungi: moving beyond the yeast paradigm [Academic Press]. In *Fungal Genetics and Biology* (Vol. 41, Issue 4). <https://doi.org/10.1016/J.FGB.2003.11.007>
- Harris, S. D., Read, N. D., Roberson, R. W., Shaw, B., Seiler, S., Plamann, M., & Momany, M. (2005). Polarisome meets spitzenkörper: microscopy, genetics, and genomics converge. *Eukaryotic Cell*, 4(2), 225–229. <https://doi.org/10.1128/EC.4.2.225-229.2005>
- Harris, S. D., Turner, G., Meyer, V., Espeso, E. A., Specht, T., Takeshita, N., & Helmstedt, K. (2009). Morphology and development in *Aspergillus nidulans*: A complex puzzle [Academic Press]. In *Fungal Genetics and Biology* (Vol. 46, Issue 1). <https://doi.org/10.1016/J.FGB.2008.07.023>
- Hérivaux, A., So, Y.-S., Gastebois, A., Latgé, J.-P., Bouchara, J.-P., Bahn, Y.-S., & Papon, N. (2016).

- Major Sensing Proteins in Pathogenic Fungi: The Hybrid Histidine Kinase Family [Public Library of Science]. In D. A. Hogan (Ed.), *PLOS Pathogens* (Vol. 12, Issue 7).
<https://doi.org/10.1371/journal.ppat.1005683>
- Hermann, T. N., & Podkovyrov, V. N. (2010). A Discovery of Riphean Heterotrophs in the Lakhanda Group of Siberia. In *Paleontological Journal* (Vol. 44, Issue 4).
<https://doi.org/10.1134/S0031030110040027>
- Herron, M. D., Borin, J. M., Boswell, J. C., Walker, J., Chen, I. C. K., Knox, C. A., Boyd, M., Rosenzweig, F., & Ratcliff, W. C. (2019). De novo origins of multicellularity in response to predation [Springer US]. In *Scientific Reports* (Vol. 9, Issue 1). <https://doi.org/10.1038/s41598-019-39558-8>
- Hope, H., Bogliolo, S., Arkowitz, R. A., & Bassilana, M. (2008). Activation of Rac1 by the Guanine Nucleotide Exchange Factor Dck1 Is Required for Invasive Filamentous Growth in the Pathogen *Candida albicans*. *Molecular Biology of the Cell*, 19(9), 3638–3651.
<https://doi.org/10.1091/mbc.e07-12-1272>
- Hughes, A. L., & Nei, M. (1989). Evolution of the major histocompatibility complex: independent origin of nonclassical class I genes in different groups of mammals. In *Molecular Biology and Evolution* (Vol. 6, Issue 6). <https://doi.org/10.1093/oxfordjournals.molbev.a040573>
- Igbalajobi, O. A., Yu, J. H., & Shin, K. S. (2017). Characterization of the *rax1* gene encoding a putative regulator of G protein signaling in *Aspergillus fumigatus* [Elsevier Ltd]. In *Biochemical and Biophysical Research Communications* (Vol. 487, Issue 2).
<https://doi.org/10.1016/j.bbrc.2017.04.079>
- Ihaka, R., & Gentleman, R. (1996). R: A Language for Data Analysis and Graphics. *Journal of Computational and Graphical Statistics*, 5(3), 299–314.
<https://doi.org/10.1080/10618600.1996.10474713>
- Jacob, F. (1977). Evolution and tinkering. *Science (New York, N.Y.)*, 196(4295), 1161–1166.
- James, T. Y., Kauff, F., Schoch, C. L., Matheny, P. B., Hofstetter, V., Cox, C. J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H. T., Rauhut, A., Reeb, V., Arnold, A. E., Amtoft, A., Stajich, J. E., Hosaka, K., Sung, G. H., Johnson, D., ... Vilgalys, R. (2006). Reconstructing the early evolution of Fungi using a six-gene phylogeny. In *Nature* (Vol. 443, Issue 7113).
<https://doi.org/10.1038/nature05110>
- James, T. Y., Porter, T. M. & Martin, W. W. (2014). 7 Blastocladiomycota. In *The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research. VII. Systematics and Evolution Part A*. Springer Berlin Heidelberg, Berlin, Heidelberg.)
- Jedd, G. (2011). Fungal evo-devo: organelles and multicellular complexity. In *Trends Cell Biol* (Vol. 21, Issue 1). <https://doi.org/10.1016/j.tcb.2010.09.001>
- Jindrich, K., & Degnan, B. M. (2016). The diversification of the basic leucine zipper family in eukaryotes correlates with the evolution of multicellularity Genome evolution and evolutionary systems biology [BMC Evolutionary Biology]. In *BMC Evolutionary Biology* (Vol. 16, Issue 1).
<https://doi.org/10.1186/s12862-016-0598-z>
- Johnson, C., Kweon, H. K., Sheidy, D., Shively, C. A., Mellacheruvu, D., Nesvizhskii, A. I., Andrews, P. C., & Kumar, A. (2014). The Yeast Sks1p Kinase Signaling Network Regulates Pseudohyphal Growth and Glucose Response. *PLoS Genetics*, 10(3), e1004183.
<https://doi.org/10.1371/journal.pgen.1004183>
- Johnson, D. I. (1999). Cdc42: An essential Rho-type GTPase controlling eukaryotic cell polarity. *Microbiology and Molecular Biology Reviews : MMBR*, 63(1), 54–105.
<http://www.ncbi.nlm.nih.gov/pubmed/10066831>

- Jorde, S., Walther, A., & Wendland, J. (2011). The *Ashbya gossypii* fimbrin SAC6 is required for fast polarized hyphal tip growth and endocytosis [Urban & Fischer]. In *Microbiological Research* (Vol. 166, Issue 3). <https://doi.org/10.1016/J.MICRES.2010.09.003>
- Jung, S. I., Rodriguez, N., Irrizary, J., Liboro, K., Bogarin, T., Macias, M., Eivers, E., Porter, E., Filler, S. G., & Park, H. (2017). Yeast casein kinase 2 governs morphology, biofilm formation, cell wall integrity, and host cell damage of *Candida albicans*. In *PLoS ONE* (Vol. 12, Issue 11). <https://doi.org/10.1371/journal.pone.0187721>
- Kaiser, D. (2001). Building a Multicellular Organism. *Annual Review of Genetics*, 35(1), 103–123. <https://doi.org/10.1146/annurev.genet.35.102401.090145>
- Katoh, K. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. In *Nucleic Acids Research* (Vol. 30, Issue 14). <https://doi.org/10.1093/nar/gkf436>
- Keeling, P. J., & Fast, N. M. (2002). Microsporidia: Biology and Evolution of Highly Reduced Intracellular Parasites. In *Annual Review of Microbiology* (Vol. 56, Issue 1). <https://doi.org/10.1146/annurev.micro.56.012302.160854>
- Kempf, M., Cottin, J., Licznar, P., Lefrançois, C., Robert, R., & Afaire-Marchais, V. (2009). Disruption of the GPI Protein-Encoding Gene IFF4 of *Candida albicans* Results in Decreased Adherence and Virulence [Springer Netherlands]. In *Mycopathologia* (Vol. 168, Issue 2). <https://doi.org/10.1007/s11046-009-9201-0>
- Kenrick, P., Wellman, C. H., Schneider, H., & Edgecombe, G. D. (2012). A timeline for terrestrialization: Consequences for the carbon cycle in the Palaeozoic. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1588), 519–536. <https://doi.org/10.1098/rstb.2011.0271>
- Kidston, R., & Lang W.H. (1921). On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part V. The Thallophyta occurring in the peat-bed; the succession of the plants throughout a vertical section of the bed, and the conditions of accumulation and preservation of the deposit. *Transactions of the Royal Society of Edinburgh* 52: 855–902.
- Kim, J., & Sudbery, P. (2011). *Candida albicans*, a major human fungal pathogen. *Journal of Microbiology*, 49(2), 171–177. <https://doi.org/10.1007/s12275-011-1064-7>
- King, N., & Carroll, S. B. (2001). A receptor tyrosine kinase from choanoflagellates: Molecular insights into early animal evolution. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 98, Issue 26). <https://doi.org/10.1073/pnas.261477698>
- King, N. (2004). The unicellular ancestry of animal development. In *Dev Cell* (2004/09/15, Vol. 7, Issue 3). <https://doi.org/10.1016/j.devcel.2004.08.010>
- King, N., Hittinger, C. T., & Carroll, S. B. (2003). Evolution of key cell signaling and adhesion protein families predates animal origins. In *Science* (Vol. 301, Issue 5631). <https://doi.org/10.1126/science.1083853>
- King, N., Westbrook, M. J., Young, S. L., Kuo, A., Abedin, M., Chapman, J., Fairclough, S., Hellsten, U., Isogai, Y., Letunic, I., Marr, M., Pincus, D., Putnam, N., Rokas, A., Wright, K. J., Zuzow, R., Dirks, W., Good, M., Goodstein, D., ... Rokhsar, D. (2008). The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. In *Nature* (Vol. 451, Issue 7180). <https://doi.org/10.1038/nature06617>
- Kirk, D. L. (1998). *Volvox: Molecular-Genetic Origins of Multicellularity*. <http://books.google.com/books?hl=en&lr=&id=79TvF1Bnak0C&oi=fnd&pg=PR13&ots=5MdedfDcsA&sig=UJgdTod8l2iMev2ApoxqY3zCH-c>
- Kirk, D. L. (2005). A twelve-step program for evolving multicellularity and a division of labor. *BioEssays*, 27(3), 299–310. <https://doi.org/10.1002/bies.20197>

- Klasson, H., Fink, G. R., & Ljungdahl, P. O. (1999). Ssy1p and Ptr3p Are Plasma Membrane Components of a Yeast System That Senses Extracellular Amino Acids. In *Molecular and Cellular Biology* (Vol. 19, Issue 8). <https://doi.org/10.1128/mcb.19.8.5405>
- Klis, F. M., Ram, A. F. J., & De Groot, P. W. J. (2007). A Molecular and Genomic View of the Fungal Cell Wall. In *Biology of the Fungal Cell*. https://doi.org/10.1007/978-3-540-70618-2_4
- Kloepper, T. H., Kienle, C. N., & Fasshauer, D. (2008). SNAREing the basis of multicellularity: Consequences of protein family expansion during evolution. In *Molecular Biology and Evolution* (Vol. 25, Issue 9). <https://doi.org/10.1093/molbev/msn151>
- Knoll, A. H., Javaux, E. J., Hewitt, D., & Cohen, P. (2006). Eukaryotic organisms in Proterozoic oceans. In *Philosophical Transactions of the Royal Society B: Biological Sciences* (Vol. 361, Issue 1470). <https://doi.org/10.1098/rstb.2006.1843>
- Knoll, A. H., & Hewitt, D. (2011). Phylogenetic, functional and ecological perspectives in complex multicellularity. In *Major transitions in evolution revisited*. MIT Press.
- Knoll, A. H. (2011). The Multiple Origins of Complex Multicellularity [Annual Reviews]. In *Annual Review of Earth and Planetary Sciences* (Vol. 39, Issue 1). <https://doi.org/10.1146/annurev.earth.031208.100209>
- Koenig S.G. & Nedelcu A.M. (2016). The mechanistic basis for the evolution of soma during the transition to multicellularity in the volvocine algae. In Newman SA and Niklas K (eds) Multicellularity: Origins and Evolution. Pp. 43-70. Vienna Series in Theoretical Biology. MIT Press.
- Kollmar, M., Lbik, D., & Enge, S. (2012). Evolution of the eukaryotic ARP2/3 activators of the WASP family: WASP, WAVE, WASH, and WHAMM, and the proposed new family members WAWH and WAML. *BMC Research Notes*, 5(1), 88. <https://doi.org/10.1186/1756-0500-5-88>
- Kornitzer, D. (2019). Regulation of candida albicans hyphal morphogenesis by endogenous signals. In *Journal of Fungi* (Vol. 5, Issue 1). <https://doi.org/10.3390/jof5010021>
- Koyanagi, M., Suga, H., Hoshiyama, D., Ono, K., Iwabe, N., Kuma, K. I., & Miyata, T. (1998). Ancient gene duplication and domain shuffling in the animal cyclic nucleotide phosphodiesterase family. In *FEBS Letters* (Vol. 436, Issue 3). [https://doi.org/10.1016/S0014-5793\(98\)01150-8](https://doi.org/10.1016/S0014-5793(98)01150-8)
- Krantz, M., Becit, E. & Hohmann, S. Comparative genomics of the HOG-signalling system in fungi. *Curr Genet* **49**, 137–151 (2006). <https://doi.org/10.1007/s00294-005-0038-x>
- Krizsán, K., Almási, É., Merényi, Z., Sahu, N., Virágh, M., Kószó, T., Mondo, S., Kiss, B., Bálint, B., Kües, U., Barry, K., Cseklye, J., Hegedüs, B., Henrissat, B., Johnson, J., Lipzen, A., Ohm, R. A., Nagy, I., Pangilinan, J., ... Nagy, L. G. (2019). Transcriptomic atlas of mushroom development reveals conserved genes behind complex multicellularity in fungi. *Proceedings of the National Academy of Sciences of the United States of America*, 116(15), 7409–7418. <https://doi.org/10.1073/pnas.1817822116>
- Kües, U., Khonsuntia, W., & Subba, S. (2018). Complex fungi [Elsevier]. In *Fungal Biology Reviews* (Vol. 32, Issue 4). <https://doi.org/10.1016/J.FBR.2018.08.001>
- Kulkarni, R. D., Thon, M. R., Pan, H., & Dean, R. A. (2005). Novel G-protein-coupled receptor-like proteins in the plant pathogenic fungus *Magnaporthe grisea* [BioMed Central]. In *Genome Biology* (Vol. 6, Issue 3). <https://doi.org/10.1186/gb-2005-6-3-r24>
- Lafon, A., Han, K.-H., Seo, J.-A., Yu, J.-H., & d'Enfert, C. (2006). G-protein and cAMP-mediated signaling in aspergilli: A genomic perspective [Academic Press]. In *Fungal Genetics and Biology* (Vol. 43, Issue 7). <https://doi.org/10.1016/J.FGB.2006.02.001>
- Lakämper, S., Kallipolitou, A., Woehlke, G., Schliwa, M., & Meyhöfer, E. (2003). Single fungal kinesin motor molecules move processively along microtubules. *Biophysical Journal*, 84(3), 1833–1843.

[https://doi.org/10.1016/S0006-3495\(03\)74991-1](https://doi.org/10.1016/S0006-3495(03)74991-1)

- Lalucque, H., & Silar, P. (2003). NADPH oxidase: An enzyme for multicellularity? In *Trends in Microbiology* (Vol. 11, Issue 1). [https://doi.org/10.1016/S0966-842X\(02\)00007-0](https://doi.org/10.1016/S0966-842X(02)00007-0)
- Larroux, C., Luke, G. N., Koopman, P., Rokhsar, D. S., Shimeld, S. M., & Degnan, B. M. (2008). Genesis and expansion of metazoan transcription factor gene classes. In *Molecular Biology and Evolution* (Vol. 25, Issue 5). <https://doi.org/10.1093/molbev/msn047>
- Laundon, D., Christmas, N., Wheeler, G., & Cunliffe, M. (2019). Chytrid rhizoid morphogenesis is adaptive and resembles hyphal development in 'higher' fungi. *BioRxiv*, 735381. <https://doi.org/10.1101/735381>
- Lee, S. C., Schmidtke, S. N., Dangott, L. J., & Shaw, B. D. (2008). *Aspergillus nidulans* ArfB plays a role in endocytosis and polarized growth. *Eukaryotic Cell*, 7(8), 1278–1288. <https://doi.org/10.1128/EC.00039-08>
- Leeder, A. C., & Turner, G. (2008). Characterisation of *Aspergillus nidulans* polarisome component BemA. In *Fungal Genetics and Biology* (Vol. 45, Issue 6). <https://doi.org/10.1016/j.fgb.2007.12.001>
- Levin, D. E. (2005). Cell wall integrity signaling in *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews : MMBR*, 69(2), 262–291. <https://doi.org/10.1128/MMBR.69.2.262-291.2005>
- Lew, R. R. (2011). How does a hypha grow? The biophysics of pressurized growth in fungi. In *Nat Rev Microbiol* (2011/06/07, Vol. 9, Issue 7). <https://doi.org/10.1038/nrmicro2591>
- Li, S., Du, L., Yuen, G., & Harris, S. D. (2006). Distinct Ceramide Synthases Regulate Polarized Growth in the Filamentous Fungus *Aspergillus nidulans*. *Molecular Biology of the Cell*, 17(3), 1218–1227. <https://doi.org/10.1091/mbc.e05-06-0533>
- Lichius, A., Yáñez-Gutiérrez, M. E., Read, N. D., & Castro-Longoria, E. (2012). Comparative Live-Cell Imaging Analyses of SPA-2, BUD-6 and BNI-1 in *Neurospora crassa* Reveal Novel Features of the Filamentous Fungal Polarisome. *PLoS ONE*, 7(1), e30372. <https://doi.org/10.1371/journal.pone.0030372>
- Lim, W. A., & Pawson, T. (2010). Phosphotyrosine Signaling: Evolving a New Cellular Communication System [Cell Press]. In *Cell* (Vol. 142, Issue 5). <https://doi.org/10.1016/J.CELL.2010.08.023>
- Lin, X., Alspaugh, J. A., Liu, H., & Harris, S. (2014). Fungal morphogenesis. *Cold Spring Harbor Perspectives in Medicine*, 5(2), a019679. <https://doi.org/10.1101/cshperspect.a019679>
- Lindsey, R., Cowden, S., Hernández-Rodríguez, Y., & Momany, M. (2010). Septins AspA and AspC are important for normal development and limit the emergence of new growth foci in the multicellular fungus *Aspergillus nidulans*. *Eukaryotic Cell*, 9(1), 155–163. <https://doi.org/10.1128/EC.00269-09>
- Loh, K. M., van Amerongen, R., & Nusse, R. (2016). Generating Cellular Diversity and Spatial Form: Wnt Signaling and the Evolution of Multicellular Animals. In *Developmental Cell* (Vol. 38, Issue 6). <https://doi.org/10.1016/j.devcel.2016.08.011>
- Lorenz, M. C., & Heitman, J. (1998). The MEP2 ammonium permease regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. *The EMBO Journal*, 17(5), 1236–1247. <https://doi.org/10.1093/EMBOJ/17.5.1236>
- Loron, C. C., François, C., Rainbird, R. H., Turner, E. C., Borensztajn, S., & Javaux, E. J. (2019). Early fungi from the Proterozoic era in Arctic Canada. In *Nature* (Vol. 570, Issue 7760). <https://doi.org/10.1038/s41586-019-1217-0>
- Löytynoja, A. (2014). *Phylogeny-aware alignment with PRANK* (pp. 155–170). Humana Press, Totowa,

- NJ. https://doi.org/10.1007/978-1-62703-646-7_10
- Lozada-Chávez, I., Stadler, P. F., & Prohaska, S. J. (2018). Genome-wide features of introns are evolutionary decoupled among themselves and from genome size throughout Eukarya. *BioRxiv*, 283549. <https://doi.org/10.1101/283549>
- Lucas, W. J., Ham, B. K., & Kim, J. Y. (2009). Plasmodesmata - bridging the gap between neighboring plant cells. In *Trends in Cell Biology* (Vol. 19, Issue 10). <https://doi.org/10.1016/j.tcb.2009.07.003>
- Lussier, M., Sdicu, A. M., Shahinian, S., & Bussey, H. (1998). The *Candida albicans* KRE9 gene is required for cell wall beta-1, 6-glucan synthesis and is essential for growth on glucose. *Proceedings of the National Academy of Sciences of the United States of America*, 95(17), 9825–9830. <https://doi.org/10.1073/PNAS.95.17.9825>
- Lynch, M. (2002). Genomics: Gene duplication and evolution. In *Science* (Vol. 297, Issue 5583). <https://doi.org/10.1126/science.1075472>
- Lynch, M., & Conery, J. S. (2000). The Evolutionary Fate and Consequences of Duplicate Genes. *Science*, 290(5494), 1151 LP – 1155. <https://doi.org/10.1126/science.290.5494.1151>
- Ma, D., & Li, R. (2013). Current Understanding of HOG-MAPK Pathway in *Aspergillus fumigatus*. *Mycopathologia*, 175(1–2), 13–23. <https://doi.org/10.1007/s11046-012-9600-5>
- Mähs, A., Ischebeck, T., Heilig, Y., Stenzel, I., Hempel, F., Seiler, S., & Heilmann, I. (2012). The Essential Phosphoinositide Kinase MSS-4 Is Required for Polar Hyphal Morphogenesis, Localizing to Sites of Growth and Cell Fusion in *Neurospora crassa* [Public Library of Science]. In A. Herrera-Estrella (Ed.), *PLoS ONE* (Vol. 7, Issue 12). <https://doi.org/10.1371/journal.pone.0051454>
- Mao, Y., & Finnemann, S. C. (2015). Regulation of phagocytosis by Rho GTPases. *Small GTPases*, 6(2), 89. <https://doi.org/10.4161/21541248.2014.989785>
- Marijuán, P. C., del Moral, R., & Navarro, J. (2013). On eukaryotic intelligence: Signaling system's guidance in the evolution of multicellular organization [Elsevier Ireland Ltd]. In *BioSystems* (Vol. 114, Issue 1). <https://doi.org/10.1016/j.biosystems.2013.06.005>
- Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, 14(12), 760–773. <https://doi.org/10.1038/nrmicro.2016.149>
- May, R. C., Caron, E., Hall, A., & Machesky, L. M. (2000). Involvement of the Arp2/3 complex in phagocytosis mediated by FcγR or CR3 [Nature Publishing Group]. In *Nature Cell Biology* (Vol. 2, Issue 4). <https://doi.org/10.1038/35008673>
- McDaniel, D. P., & Roberson, R. W. (1998). PROTOPtASMA -Tubulin is a component of the Spitzenkörper and centrosomes in hyphal-tip cells of *Allomyces macrogynus* Rapid Communication. In *Protoplasma*.
- McLennan, D. A. (2008). The Concept of Co-option; Why Evolution Often Looks Miraculous. *Evo Edu Outreach*, 1:247-258. <https://doi.org/10.1007/s12052-008-0053-8>
- McGoldrick, C. A., Gruver, C., & May, G. S. (1995). myoA of *Aspergillus nidulans* encodes an essential myosin I required for secretion and polarized growth. *The Journal of Cell Biology*, 128(4), 577–587. <https://doi.org/10.1083/JCB.128.4.577>
- Medina, M., Collins, A. G., Taylor, J. W., Valentine, J. W., Lipps, J. H., Amaral-Zettler, L., & Sogin, M. L. (2003). Phylogeny of Opisthokonta and the evolution of multicellularity and complexity in Fungi and Metazoa. In *International Journal of Astrobiology* (Vol. 2, Issue 3). <https://doi.org/10.1017/S1473550403001551>
- Mendoza, A. de, Sebé-Pedrós, A., Šestak, M. S., Matejčić, M., Torruella, G., Domazet-Lošo, T., & Ruiz-

- Trillo, I. (2013). Transcription factor evolution in eukaryotes and the assembly of the regulatory toolkit in multicellular lineages. *Proceedings of the National Academy of Sciences*, 110(50), E4858–E4866. <https://doi.org/10.1073/PNAS.1311818110>
- Merson-Davies, L. A., & Odds, F. C. (1989). A morphology index for characterization of cell shape in *Candida albicans*. In *Journal of General Microbiology* (Vol. 135, Issue 11). <https://doi.org/10.1099/00221287-135-11-3143>
- Michod, R. E., & Roze, D. (2001). Cooperation and conflict in the evolution of multicellularity. In *Heredity* (Vol. 86, Issue 1). <https://doi.org/10.1046/j.1365-2540.2001.00808.x>
- Michod, R. E., Nedelcu, A. M., & Roze, D. (2003). Cooperation and conflict in the evolution of individuality: IV. Conflict mediation and evolvability in *Volvox carteri*. *Biosystems*, 69(2), 95–114. [https://doi.org/https://doi.org/10.1016/S0303-2647\(02\)00133-8](https://doi.org/https://doi.org/10.1016/S0303-2647(02)00133-8)
- Miller, K. Y., Wu, J., & Miller, B. L. (1992). StuA is required for cell pattern formation in *Aspergillus*. In *Genes and Development* (Vol. 6, Issue 9). <https://doi.org/10.1101/gad.6.9.1770>
- Miller, W. T. (2012). Tyrosine kinase signaling and the emergence of multicellularity. *Biochimica et Biophysica Acta*, 1823(6), 1053–1057. <https://doi.org/10.1016/j.bbamcr.2012.03.009>
- Momany, M. (2002). Polarity in filamentous fungi: establishment, maintenance and new axes. *Current Opinion in Microbiology*, 5(6), 580–585. [https://doi.org/10.1016/S1369-5274\(02\)00368-5](https://doi.org/10.1016/S1369-5274(02)00368-5)
- Moreno-Ruiz, E., Ortu, G., de Groot, P. W. J., Cottier, F., Loussert, C., Prevost, M.-C., de Koster, C., Klis, F. M., Goyard, S., & d'Enfert, C. (2009). The GPI-modified proteins Pga59 and Pga62 of *Candida albicans* are required for cell wall integrity. *Microbiology*, 155(6), 2004–2020. <https://doi.org/10.1099/mic.0.028902-0>
- Morris, N. R. (1975). Mitotic mutants of *Aspergillus nidulans*. *Genetical Research*, 26(3), 237–254. <https://doi.org/10.1017/S0016672300016049>
- Muller, H. J. (1935). Further studies on the nature and causes of gene mutations. *Proc. Sixth Int. Congr. Genet.* 1:213-255
- Muller, H. J. (1936). *Bar* duplication. *Science* 83(2161):528-530.
- Muszkiet, L., Aïmanianda, V., Mellado, E., Gribaldo, S., Alcázar-Fuoli, L., Szewczyk, E., Prevost, M.-C., & Latgé, J.-P. (2014). Deciphering the role of the chitin synthase families 1 and 2 in the *in vivo* and *in vitro* growth of *Aspergillus fumigatus* by multiple gene targeting deletion. *Cellular Microbiology*, 16(12), 1784–1805. <https://doi.org/10.1111/cmi.12326>
- Nadal, M., García-Pedrajas, M. D., & Gold, S. E. (2008). Dimorphism in fungal plant pathogens. In *FEMS Microbiology Letters* (Vol. 284, Issue 2). <https://doi.org/10.1111/j.1574-6968.2008.01173.x>
- Nagy, L. G. (2017). Evolution: Complex Multicellular Life with 5,500 Genes [Elsevier Ltd]. In *Current Biology* (Vol. 27, Issue 12). <https://doi.org/10.1016/j.cub.2017.04.032>
- Nagy, L. G., Ohm, R. A., Kovács, G. M., Floudas, D., Riley, R., Gácsér, A., Sipiczki, M., Davis, J. M., Doty, S. L., de Hoog, G. S., Lang, B. F., Spatafora, J. W., Martin, F. M., Grigoriev, I. V., & Hibbett, D. S. (2014). Latent homology and convergent regulatory evolution underlies the repeated emergence of yeasts [Nature Publishing Group]. In *Nature Communications* (Vol. 5, Issue 1). <https://doi.org/10.1038/ncomms5471>
- Nagy, L. G., Kovács, G. M., & Krizsán, K. (2018). Complex multicellularity in fungi: evolutionary convergence, single origin, or both? [Wiley/Blackwell (10.1111)]. In *Biological Reviews*. <https://doi.org/10.1111/brv.12418>
- Nagy, L. G., Riley, R., Tritt, A., Adam, C., Daum, C., Floudas, D., Sun, H., Yadav, J. S., Pangilinan, J., Larsson, K. H., Matsuura, K., Barry, K., Labutti, K., Kuo, R., Ohm, R. A., Bhattacharya, S. S.,

- Shirouzu, T., Yoshinaga, Y., Martin, F. M., ... Hibbett, D. S. (2016). Comparative genomics of early-diverging mushroom-forming fungi provides insights into the origins of lignocellulose decay capabilities. *Molecular Biology and Evolution*, 33(4), 959–970. <https://doi.org/10.1093/molbev/msv337>
- Nagy, L. G., Tóth, R., Kiss, E., Slot, J., Gácsér, A., & Kovács, G. M. (2017). Six Key Traits of Fungi: Their Evolutionary Origins and Genetic Bases [American Society of Microbiology]. In *The Fungal Kingdom* (Vol. 5, Issue 4). <https://doi.org/10.1128/microbiolspec.FUNK-0036-2016>
- Nam, J., Kim, J., Lee, S., An, G., Ma, H., & Nei, M. (2004). Type I MADS-box genes have experienced faster birth-and-death evolution than type II MADS-box genes in angiosperms. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 101, Issue 7). <https://doi.org/10.1073/pnas.0308430100>
- Naranjo-Ortiz, M. A., & Gabaldón, T. (2019). Fungal evolution: major ecological adaptations and evolutionary transitions. In *Biological Reviews* (Vol. 94, Issue 4). <https://doi.org/10.1111/brv.12510>
- Navarro-García, F., Alonso-Monge, R., Rico, H., Pla, J., Sentandreu, R., & Nombela, C. (1998). A role for the MAP kinase gene MKC1 in cell wall construction and morphological transitions in *Candida albicans*. In *Microbiology* (Vol. 144, Issue 2). <https://doi.org/10.1099/00221287-144-2-411>
- Nei, M., Gu, X., & Sitnikova, T. (1997). Evolution by the birth-and-death process in multigene families of the vertebrate immune system. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 94, Issue 15). <https://doi.org/10.1073/pnas.94.15.7799>
- Nei, M., & Rooney, A. P. (2005). Concerted and Birth-and-Death Evolution of Multigene Families. In *Annual Review of Genetics* (Vol. 39, Issue 1). <https://doi.org/10.1146/annurev.genet.39.073003.112240>
- Nelson, B., Kurischko, C., Horecka, J., Mody, M., Nair, P., Pratt, L., Zougman, A., McBroom, L. D. B., Hughes, T. R., Boone, C., & Luca, F. C. (2003). RAM: a conserved signaling network that regulates Ace2p transcriptional activity and polarized morphogenesis. *Molecular Biology of the Cell*, 14(9), 3782–3803. <https://doi.org/10.1091/mbc.e03-01-0018>
- Nelson, D. R., (2013). A world of cyochrome P450s. *Philos Trans R Soc Lond B Sci.* 368(1612):20120430. <https://doi.org/10.1098/rstb.2012.0430>
- Nemecek, J. C., Wüthrich, M., & Klein, B. S. (2006). Global control of dimorphism and virulence in fungi. *Science*, 312(5773), 583–588. <https://doi.org/10.1126/science.1124105>
- Nguyen, T. A., Cissé, O. H., Yun Wong, J., Zheng, P., Hewitt, D., Nowrousian, M., Stajich, J. E., & Jedd, G. (2017). Innovation and constraint leading to complex multicellularity in the Ascomycota. *Nature Communications*, 8, 14444. <https://doi.org/10.1038/ncomms14444>
- Nichols, S. A., Dirks, W., Pearse, J. S., & King, N. (2006). Early evolution of animal cell signaling and adhesion genes. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 103, Issue 33). <https://doi.org/10.1073/pnas.0604065103>
- Nichols, S. A., Roberts, B. W., Richter, D. J., Fairclough, S. R., & King, N. (2012). Origin of metazoan cadherin diversity and the antiquity of the classical cadherin/β-catenin complex. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 109, Issue 32). <https://doi.org/10.1073/pnas.1120685109>
- Nickerson, K. W., Atkin, A. L., & Hornby, J. M. (2006). Quorum Sensing in Dimorphic Fungi: Farnesol and Beyond. *Appl. Environ. Microbiol.*, 72(6), 3805–3813. <https://doi.org/10.1128/AEM.02765-05>
- Niklas, K. J., Cobb, E. D., & Crawford, D. R. (2013). The evo-devo of multinucleate cells, tissues, and organisms, and an alternative route to multicellularity. *Evol Dev*, 15(6), 466–474. <https://doi.org/10.1111/ede.12055>

- Niklas, K. J., & Newman, S. A. (2013). The origins of multicellular organisms. In *Evol Dev* (2013/01/22, Vol. 15, Issue 1). <https://doi.org/10.1111/ede.12013>
- Niklas, K. J. (2014). The evolutionary-developmental origins of multicellularity. In *American Journal of Botany* (Vol. 101, Issue 1). <https://doi.org/10.3732/ajb.1300314>
- O'Malley, M. A., Wideman, J. G., & Ruiz-Trillo, I. (2016). Losing Complexity: The Role of Simplification in Macroevolution. *Trends Ecol Evol*, 31(8), 608–621. <https://doi.org/10.1016/j.tree.2016.04.004>
- Omura, T. (2013). Contribution of cytochrome P450 to the diversification of eukaryotic organisms. *Biotechnol Appl Biochem*. 60(1):4-8. <https://doi.org/10.1002/bab.1099>
- Ohm, R. A., Feau, N., Henrissat, B., Schoch, C. L., Horwitz, B. A., Barry, K. W., Condon, B. J., Copeland, A. C., Dhillon, B., Glaser, F., Hesse, C. N., Kosti, I., LaButti, K., Lindquist, E. A., Lucas, S., Salamov, A. A., Bradshaw, R. E., Ciuffetti, L., Hamelin, R. C., ... Grigoriev, I. V. (2012). Diverse Lifestyles and Strategies of Plant Pathogenesis Encoded in the Genomes of Eighteen Dothideomycetes Fungi [Public Library of Science]. In A. Andrianopoulos (Ed.), *PLoS Pathogens* (Vol. 8, Issue 12). <https://doi.org/10.1371/journal.ppat.1003037>
- Ohno, S. (1970). *Evolution by Gene Duplication*. Springer, Berlin.
- Ohno, S., Wolf, U., & Atkin, N. B. (1968). Evolution From Fish To Mammals By Gene Duplication. In *Hereditas* (Vol. 59, Issue 1). <https://doi.org/10.1111/j.1601-5223.1968.tb02169.x>
- Orci, L., Palmer, D. J., Ravazzola, M., Perrelet, A., Amherdt, M., Rothman, J. E. (1993). Budding from Golgi membranes requires the coatamer complex of non-clathrin coat proteins. *Nature*, 362(6421), 648-652. <https://doi.org/10.1038/362648a0>
- Ordaz-Ortiz, J. J., Marcus, S. E., & Paul Knox, J. (2009). Cell wall microstructure analysis implicates hemicellulose polysaccharides in cell adhesion in tomato fruit pericarp parenchyma. In *Molecular Plant* (Vol. 2, Issue 5). <https://doi.org/10.1093/mp/ssp049>
- Palecek, S. P., Kron, S. J., & Parikh, A. S. (2002). Sensing, signalling and integrating physical processes during *Saccharomyces cerevisiae* invasive and filamentous growth. *Microbiology*, 148(4), 893–907. <https://doi.org/10.1099/00221287-148-4-893>
- Park, G., Servin, J. A., Turner, G. E., Altamirano, L., Colot, H. V., Collopy, P., Litvinkova, L., Li, L., Jones, C. A., Diala, F.-G., Dunlap, J. C., & Borkovich, K. A. (2011). Global Analysis of Serine-Threonine Protein Kinase Genes in *Neurospora crassa*. In *Eukaryotic Cell* (Vol. 10, Issue 11). <https://doi.org/10.1128/EC.05140-11>
- Peberdy, J. F. (1994). Protein secretion in filamentous fungi - trying to understand a highly productive black box. In *Trends in Biotechnology* (Vol. 12, Issue 2). [https://doi.org/10.1016/0167-7799\(94\)90100-7](https://doi.org/10.1016/0167-7799(94)90100-7)
- Peñalva, M. A. (2010). Endocytosis in filamentous fungi: Cinderella gets her reward. *Current Opinion in Microbiology*, 13(6), 684–692. <https://doi.org/10.1016/J.MIB.2010.09.005>
- Peñalva, M. A., Galindo, A., Abenza, J. F., Pinar, M., Calcagno-Pizarelli, A. M., Arst, H. N., & Pantazopoulou, A. (2012). Searching for gold beyond mitosis. *Cellular Logistics*, 2(1), 2–14. <https://doi.org/10.4161/cl.19304>
- Pentz, J. T., Márquez-Zacarias, P., Yunker, P. J., Libby, E., & Ratcliff, W. C. (2018). Ecological advantages and evolutionary limitations of aggregative multicellular development. *BioRxiv*, 255307. <https://doi.org/10.1101/255307>
- Philippsen, P., Kaufmann, A., & Schmitz, H.-P. (2005). Homologues of yeast polarity genes control the development of multinucleated hyphae in *Ashbya gossypii* [Elsevier Current Trends]. In *Current Opinion in Microbiology* (Vol. 8, Issue 4). <https://doi.org/10.1016/J.MIB.2005.06.021>

- Piontkivska, H., Rooney, A. P., & Nei, M. (2002). Purifying selection and birth-and-death evolution in the histone H4 gene family. In *Molecular Biology and Evolution* (Vol. 19, Issue 5). <https://doi.org/10.1093/oxfordjournals.molbev.a004127>
- Pirozynski, K. A., & Malloch, D. W. (1975). The origin of land plants: A matter of mycotrophism. *Biosystems*, 6(3), 153–164. [https://doi.org/10.1016/0303-2647\(75\)90023-4](https://doi.org/10.1016/0303-2647(75)90023-4)
- Pöggeler, S., Nowrousian, M., Teichert, I., Beier, A., & Kück, U. (2018). Fruiting-Body Development in Ascomycetes. In *Physiology and Genetics* (pp. 1–56). Springer International Publishing. https://doi.org/10.1007/978-3-319-71740-1_1
- Prasanna, A. N., Gerber, D., Kijpornyongpan, T., Aime, M. C., Doyle, V. P., & Nagy, L. G. (2019). Model Choice, Missing Data and Taxon Sampling Impact Phylogenomic Inference of Deep Basidiomycota Relationships. *Systematic Biology*. <https://doi.org/10.1093/sysbio/syz029>
- Pressel, S., Bidartondo, M. I., Ligrone, R., & Duckett, J. G. (2010). *Phytotaxa, Fungal symbioses in bryophytes: New insights in the Twenty First Century*. 253, 4. www.mapress.com/phytotaxa/
- Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Molecular Biology and Evolution*, 26(7), 1641–1650. <https://doi.org/10.1093/molbev/msp077>
- Prochnik, S. E., Umen, J., Nedelcu, A. M., Hallmann, A., Miller, S. M., Nishii, I., Ferris, P., Kuo, A., Mitros, T., Fritz-Laylin, L. K., Hellsten, U., Chapman, J., Simakov, O., Rensing, S. A., Terry, A., Pangilinan, J., Kapitonov, V., Jurka, J., Salamov, A., ... Rokhsar, D. S. (2010). Genomic analysis of organismal complexity in the multicellular green alga *Volvox carteri*. *Science (New York, N.Y.)*, 329(5988), 223–226. <https://doi.org/10.1126/science.1188800>
- Pruyne, D., & Bretscher, A. (2000). Polarization of cell growth in yeast. II. The role of the cortical actin cytoskeleton. In *Journal of Cell Science* (Vol. 113, Issue 4).
- Radcliffe, P. A., Binley, K. M., Trevethick, J., Hall, M., & Sudbery, P. E. (1997). Filamentous growth of the budding yeast *Saccharomyces cerevisiae* induced by overexpression of the WHI2 gene. *Microbiology*, 143(6), 1867–1876. <https://doi.org/10.1099/00221287-143-6-1867>
- Ramage, G., Saville, S. P., & Thomas, D. P. (2005). Bio lms: an Update†. *Society*, 4(4), 633–638. <https://doi.org/10.1128/EC.4.4.633>
- Read, N. D., & Kalkman, E. R. (2003). Does endocytosis occur in fungal hyphae? *Fungal Genetics and Biology*, 39(3), 199–203. [https://doi.org/10.1016/S1087-1845\(03\)00045-8](https://doi.org/10.1016/S1087-1845(03)00045-8)
- Redecker, D., Kodner, R., & Graham, L. E. (2000). Glomalean fungi from the Ordovician. In *Science* (Vol. 289, Issue 5486). <https://doi.org/10.1126/science.289.5486.1920>
- Reijntj, P., Jorde, S., & Wendland, J. (2010). *Candida albicans* SH3-domain proteins involved in hyphal growth, cytokinesis, and vacuolar morphology. In *Current Genetics* (Vol. 56, Issue 4). <https://doi.org/10.1007/s00294-010-0301-7>
- Remy, W., Taylor, T. N., Hass, H., & Kerp, H. (1994a). Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences*, 91(25), 11841–11843. <https://doi.org/10.1073/pnas.91.25.11841>
- Remy, W., Taylor, T. N., & Hass, H. (1994b). Early Devonian Fungi: A Blastocladalean Fungus with Sexual Reproduction. In *American Journal of Botany* (Vol. 81, Issue 6). <https://doi.org/10.2307/2445647>
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3(2), 217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>

- Revell, L. J. (2014). Ancestral character estimation under the threshold model from quantitative genetics. In *Evolution* (Vol. 68, Issue 3). <https://doi.org/10.1111/evo.12300>
- Richard, M., Iбата-Ombetta, S., Dromer, F., Bordon-Pallier, F., Jouault, T., & Gaillardin, C. (2002). Complete glycosylphosphatidylinositol anchors are required in *Candida albicans* for full morphogenesis, virulence and resistance to macrophages. In *Molecular Microbiology* (Vol. 44, Issue 3). <https://doi.org/10.1046/j.1365-2958.2002.02926.x>
- Richards, T. A., Leonard, G., & Wideman, J. G. (2017). What Defines the “Kingdom” Fungi? *Microbiology Spectrum*, 5(3). <https://doi.org/10.1128/microbiolspec.FUNK-0044-2017>
- Richards, T. A., & Talbot, N. J. (2018). Osmotrophy. *Current Biology : CB*, 28(20), R1179–R1180. <https://doi.org/10.1016/j.cub.2018.07.069>
- Richter, D. J., Fozouni, P., Eisen, M. B., & King, N. (2018). Gene family innovation, conservation and loss on the animal stem lineage. In *eLife* (Vol. 7). <https://doi.org/10.7554/eLife.34226>
- Riquelme, M., Aguirre, J., Bartnicki-García, S., Braus, G. H., Feldbrügge, M., Fleig, U., Hansberg, W., Herrera-Estrella, A., Kämper, J., Kück, U., Mouriño-Pérez, R. R., Takeshita, N., & Fischer, R. (2018). Fungal Morphogenesis, from the Polarized Growth of Hyphae to Complex Reproduction and Infection Structures. *Microbiology and Molecular Biology Reviews : MMBR*, 82(2), e00068-17. <https://doi.org/10.1128/MMBR.00068-17>
- Riquelme, M., & Sánchez-León, E. (2014). The Spitzenkörper: A choreographer of fungal growth and morphogenesis. In *Current Opinion in Microbiology* (Vol. 20). <https://doi.org/10.1016/j.mib.2014.04.003>
- Rittenour, W. R., Si, H., & Harris, S. D. (2009). Hyphal morphogenesis in *Aspergillus nidulans* [Elsevier]. In *Fungal Biology Reviews* (Vol. 23, Issues 1–2). <https://doi.org/10.1016/J.FBR.2009.08.001>
- Rivero, F. (2008). Endocytosis and the Actin Cytoskeleton in *Dictyostelium discoideum* [Academic Press]. In *International Review of Cell and Molecular Biology* (Vol. 267). [https://doi.org/10.1016/S1937-6448\(08\)00633-3](https://doi.org/10.1016/S1937-6448(08)00633-3)
- Roberson, R. W., Saucedo, E., Maclean, D., Propster, J., Unger, B., Oneil, T. A., Parvanehgozar, K., Cavanaugh, C., & Lowry, D. (2011). The hyphal tip structure of *Basidiobolus* sp.: A zygomycete fungus of uncertain phylogeny [Elsevier Ltd]. In *Fungal Biology* (Vol. 115, Issue 6). <https://doi.org/10.1016/j.funbio.2011.02.012>
- Roberts, R. L., Mösch, H.-U., & Fink, G. R. (1997). 14-3-3 Proteins Are Essential for RAS/MAPK Cascade Signaling during Pseudohyphal Development in *S. cerevisiae*. *Cell*, 89(7), 1055–1065. [https://doi.org/10.1016/S0092-8674\(00\)80293-7](https://doi.org/10.1016/S0092-8674(00)80293-7)
- Robinson, L. C., Bradley, C., Bryan, J. D., Jerome, A., Kweon, Y., & Panek, H. R. (1999). The Yck2 Yeast Casein Kinase 1 Isoform Shows Cell Cycle-specific Localization to Sites of Polarized Growth and Is Required for Proper Septin Organization. *Molecular Biology of the Cell*, 10(4), 1077–1092. <https://doi.org/10.1091/mbc.10.4.1077>
- Rogg, L. E., Fortwendel, J. R., Juvvadi, P. R., Lilley, A., & Steinbach, W. J. (2011). The chitin synthase genes *chsA* and *chsC* are not required for cell wall stress responses in the human pathogen *Aspergillus fumigatus*. *Biochemical and Biophysical Research Communications*, 411(3), 549–554. <https://doi.org/10.1016/J.BBRC.2011.06.180>
- Rokas, A. (2008). The Origins of Multicellularity and the Early History of the Genetic Toolkit For Animal Development. In *Annual Review of Genetics* (Vol. 42, Issue 1). <https://doi.org/10.1146/annurev.genet.42.110807.091513>
- Román, E., Arana, D. M., Nombela, C., Alonso-Monge, R., & Pla, J. (2007). MAP kinase pathways as regulators of fungal virulence. *Trends in Microbiology*, 15(4), 181–190.

<https://doi.org/10.1016/J.TIM.2007.02.001>

- Ruiz-Trillo, I., Burger, G., Holland, P. W. H., King, N., Lang, B. F., Roger, A. J., & Gray, M. W. (2007). The origins of multicellularity: a multi-taxon genome initiative. In *Trends in Genetics* (Vol. 23, Issue 3). <https://doi.org/10.1016/j.tig.2007.01.005>
- Sanderfoot, A. (2007). Increases in the Number of SNARE Genes Parallels the Rise of Multicellularity among the Green Plants. In *PLANT PHYSIOLOGY* (Vol. 144, Issue 1). <https://doi.org/10.1104/pp.106.092973>
- Saputo, S., Chabrier-Rosello, Y., Luca, F. C., Kumar, A., & Krysan, D. J. (2012). The RAM network in pathogenic fungi. In *Eukaryotic Cell* (Vol. 11, Issue 6). <https://doi.org/10.1128/EC.00044-12>
- Schuchardt, I., Aßmann, D., Thines, E., Schuberth, C., & Steinberg, G. (2005). Myosin-V, Kinesin-1, and Kinesin-3 Cooperate in Hyphal Growth of the Fungus *Ustilago maydis*. *Molecular Biology of the Cell*, 16(11), 5191–5201. <https://doi.org/10.1091/mbc.e05-04-0272>
- Schultzhaus, Z., & Shaw, B. D. (2016). The flippase DnfB is cargo of fimbrin-associated endocytosis in *Aspergillus nidulans*, and likely recycles through the late Golgi. *Communicative & Integrative Biology*, 9(2), e1141843. <https://doi.org/10.1080/19420889.2016.1141843>
- Schultzhaus, Z., Yan, H., & Shaw, B. D. (2015). *Aspergillus nidulans* flippase DnfA is cargo of the endocytic collar and plays complementary roles in growth and phosphatidylserine asymmetry with another flippase, DnfB. *Molecular Microbiology*, 97(1), 18–32. <https://doi.org/10.1111/mmi.13019>
- Sebé-Pedrós, A., Ballaré, C., Parra-Acero, H., Chiva, C., Tena, J. J., Sabidó, E., Gómez-Skarmeta, J. L., Di Croce, L., & Ruiz-Trillo, I. (2016). The Dynamic Regulatory Genome of *Capsaspora* and the Origin of Animal Multicellularity. In *Cell* (Vol. 165, Issue 5). <https://doi.org/10.1016/j.cell.2016.03.034>
- Sebé-Pedrós, A., De Mendoza, A., Lang, B. F., Degnan, B. M., & Ruiz-Trillo, I. (2011). Unexpected repertoire of metazoan transcription factors in the unicellular holozoan *Capsaspora owczarzaki*. In *Molecular Biology and Evolution* (Vol. 28, Issue 3). <https://doi.org/10.1093/molbev/msq309>
- Sebé-Pedrós, A., Degnan, B. M., & Ruiz-Trillo, I. (2017). The origin of Metazoa: a unicellular perspective [Nature Publishing Group]. In *Nature Reviews Genetics* (Vol. 18, Issue 8). <https://doi.org/10.1038/nrg.2017.21>
- Sebé-Pedrós, A., Grau-Bové, X., Richards, T. A., & Ruiz-Trillo, I. (2014). Evolution and Classification of Myosins, a Eukaryotic Whole-Genome Approach. *Genome Biology and Evolution*, 6(2), 290–305. <https://doi.org/10.1093/gbe/evu013>
- Sebé-Pedrós, A., Irimia, M., del Campo, J., Parra-Acero, H., Russ, C., Nusbaum, C., Blencowe, B. J., & Ruiz-Trillo, I. (2013). Regulated aggregative multicellularity in a close unicellular relative of metazoa [eLife Sciences Publications, Ltd]. In D. Tautz (Ed.), *Elife* (Vol. 2). <https://doi.org/10.7554/eLife.01287>
- Sebé-Pedrós, A., Roger, A. J., Lang, B. F., King, N., & Ruiz-Trillo, I. (2010). Ancient origin of the integrin-mediated adhesion and signaling machinery. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 107, Issue 22). <https://doi.org/10.1073/pnas.1002257107>
- Sebé-Pedrós, A., Zheng, Y., Ruiz-Trillo, I., & Pan, D. (2012). Premetazoan Origin of the Hippo Signaling Pathway. In *Cell Reports* (Vol. 1, Issue 1). <https://doi.org/10.1016/j.celrep.2011.11.004>
- Sekimoto, S., Rochon, D., Long, J. E., Dee, J. M., & Berbee, M. L. (2011). A multigene phylogeny of *Olpidium* and its implications for early fungal evolution [BioMed Central]. In *BMC Evolutionary Biology* (Vol. 11, Issue 1). <https://doi.org/10.1186/1471-2148-11-331>
- Selosse, M. A., & Tacon, F. Le. (1998). Selosse & Le The land flora: a phototroph–fungus partnership?

Tree, 13(1), 15–20.

- Selosse, M. A., Baudoin, E., & Vandenkoornhuyse, P. (2004). Symbiotic microorganisms, a key for ecological success and protection of plants. *Comptes Rendus - Biologies*, 327(7), 639–648. <https://doi.org/10.1016/j.crv.2003.12.008>
- Semighini, C. P., & Harris, S. D. (2008). Regulation of apical dominance in *Aspergillus nidulans* hyphae by reactive oxygen species. In *Genetics* (Vol. 179, Issue 4). <https://doi.org/10.1534/genetics.108.089318>
- Shapiro, J. A. (1998). Thinking About Bacterial Populations As Multicellular Organisms. In *Annual Review of Microbiology* (Vol. 52, Issue 1). <https://doi.org/10.1146/annurev.micro.52.1.81>
- Shaw, B. D., Chung, D. W., Wang, C. L., Quintanilla, L. A., & Upadhyay, S. (2011). A role for endocytic recycling in hyphal growth [Elsevier Ltd]. In *Fungal Biology* (Vol. 115, Issue 6). <https://doi.org/10.1016/j.funbio.2011.02.010>
- Sheppard, D. C., Doedt, T., Chiang, L. Y., Kim, H. S., Chen, D., Nierman, W. C., & Filler, S. G. (2005). The *Aspergillus fumigatus* StuA Protein Governs the Up-Regulation of a Discrete Transcriptional Program during the Acquisition of Developmental Competence. *Molecular Biology of the Cell*, 16(12), 5866–5879. <https://doi.org/10.1091/mbc.e05-07-0617>
- Slamovits, C. H., Fast, N. M., Law, J. S., Keeling, P. J. (2004). Genome Compaction and Stability in Microsporidian Intracellular Parasites. *Current Biology*, Vol. 14, 891–896. <https://doi.org/10.1016/j.cub.2004.04.041>
- Spatafora, J. W., Aime, M. C., Grigoriev, I. V., Martin, F., Stajich, J. E., & Blackwell, M. (2017). The Fungal Tree of Life: From Molecular Systematics to Genome-Scale Phylogenies [American Society of Microbiology]. In *The Fungal Kingdom* (Vol. 5, Issue 5). <https://doi.org/10.1128/microbiolspec.FUNK-0053-2016>
- Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., Bonito, G., Corradi, N., Grigoriev, I., Gryganskyi, A., James, T. Y., O'Donnell, K., Roberson, R. W., Taylor, T. N., Uehling, J., Vilgalys, R., White, M. M., & Stajich, J. E. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. In *Mycologia* (Vol. 108, Issue 5). <https://doi.org/10.3852/16-042>
- Spreghini, E., Davis, D. A., Subaran, R., Kim, M., & Mitchell, A. P. (2003). Roles of *Candida albicans* Dfg5p and Dcw1p cell surface proteins in growth and hypha formation. *Eukaryotic Cell*, 2(4), 746–755. <https://doi.org/10.1128/EC.2.4.746-755.2003>
- Srinivasan, S., Vargas, M. M., & Roberson, R. W. (1996). Functional, organizational, and biochemical analysis of actin in hyphal tip cells of *Allomyces macrogynus*. In *Mycologia* (Vol. 88, Issue 1). <https://doi.org/10.1080/00275514.1996.12026624>
- Stajich, J. E., Wilke, S. K., Ahren, D., Au, C. H., Birren, B. W., Borodovsky, M., Burns, C., Canback, B., Casselton, L. A., Cheng, C. K., Deng, J., Dietrich, F. S., Fargo, D. C., Farman, M. L., Gathman, A. C., Goldberg, J., Guigo, R., Hoegger, P. J., Hooker, J. B., ... Pukkila, P. J. (2010). Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). In *Proc Natl Acad Sci U S A* (2010/06/16, Vol. 107, Issue 26). <https://doi.org/1003391107> [pii]10.1073/pnas.1003391107
- Stajich, J. E., Berbee, M. L., Blackwell, M., Hibbett, D. S., James, T. Y., Spatafora, J. W., & Taylor, J. W. (2009). The Fungi [Cell Press]. In *Current Biology* (Vol. 19, Issue 18). <https://doi.org/10.1016/J.CUB.2009.07.004>
- Stamatakis, A. (2014). RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Steinberg, G. (2007a). Preparing the way: fungal motors in microtubule organization. *Trends in*

- Microbiology*, 15(1), 14–21. <https://doi.org/10.1016/J.TIM.2006.11.007>
- Steinberg, G. (2007b). Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. *Eukaryotic Cell*, 6(3), 351–360. <https://doi.org/10.1128/EC.00381-06>
- Steinberg, G. (2014). Endocytosis and early endosome motility in filamentous fungi [Elsevier Ltd]. In *Current Opinion in Microbiology* (Vol. 20). <https://doi.org/10.1016/j.mib.2014.04.001>
- Steinberg, G., Peñalva, M. A., Riquelme, M., Wosten, H., & Harris, S. D. (2017). Cell Biology of Hyphal Growth. *Microbiology Spectrum*, 5(2), 1–34. <https://doi.org/https://doi.org/10.1128/microbiolspec.funk-0034-2016>
- Steinberg, G., & Schliwa, M. (1995). The Neurospora organelle motor: A distant relative of conventional kinesin with unconventional properties. *Molecular Biology of the Cell*, 6(11), 1605–1618. <https://doi.org/10.1091/mbc.6.11.1605>
- Steinberg, G., & Schliwa, M. (1996). Characterization of the biophysical and motility properties of kinesin from the fungus *Neurospora crassa*. *Journal of Biological Chemistry*, 271(13), 7516–7521. <https://doi.org/10.1074/jbc.271.13.7516>
- Strullu-Derrien, C. (2018). Fossil filamentous microorganisms associated with plants in early terrestrial environments [Elsevier Ltd]. In *Current Opinion in Plant Biology* (Vol. 44). <https://doi.org/10.1016/j.pbi.2018.04.001>
- Strullu-Derrien, C., Kenrick, P., Pressel, S., Duckett, J. G., Rioult, J.-P., & Strullu, D.-G. (2014). Fungal associations in *Horneophyton ligneri* from the Rhynie Chert (c . 407 million year old) closely resemble those in extant lower land plants: novel insights into ancestral plant-fungus symbioses. In *New Phytologist* (Vol. 203, Issue 3). <https://doi.org/10.1111/nph.12805>
- Strullu-Derrien, C., Selosse, M. A., Kenrick, P., & Martin, F. M. (2018a). The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. In *New Phytologist* (Vol. 220, Issue 4). <https://doi.org/10.1111/nph.15076>
- Strullu-Derrien, C., Spencer, A. R. T., Goral, T., Dee, J., Honegger, R., Kenrick, P., Longcore, J. E., & Berbee, M. L. (2018b). New insights into the evolutionary history of Fungi from a 407 Ma Blastocladiomycota fossil showing a complex hyphal thallus. In *Philosophical Transactions of the Royal Society B: Biological Sciences* (Vol. 373, Issue 1739). <https://doi.org/10.1098/rstb.2016.0502>
- Strullu-Derrien, C., Wawrzyniak, Z., Goral, T., & Kenrick, P. (2015). Fungal colonization of the rooting system of the early land plant *Asteroxylon mackiei* from the 407-Myr-old Rhynie Chert (Scotland, UK). In *Botanical Journal of the Linnean Society* (Vol. 179, Issue 1). <https://doi.org/10.1111/boj.12307>
- Strullu-Derrien, C., Rioult JP, Strullu DG, Mycorrhizas in Upper Carboniferous Radiculites-type cordaitalean rootlets, New Phytologist. <https://doi.org/10.1111/j.1469-8137.2009.02805.x> Volume182, Issue3May 2009 Pages 561-564
- Sudbery, P. E. (2011). Growth of *Candida albicans* hyphae [Nature Publishing Group]. In *Nature Reviews Microbiology* (Vol. 9, Issue 10). <https://doi.org/10.1038/nrmicro2636>
- Sudbery, P., Gow, N., & Berman, J. (2004). The distinct morphogenic states of *Candida albicans*. *Trends in Microbiology*, 12(7), 317–324. <https://doi.org/10.1016/j.tim.2004.05.008>
- Suga, H., Chen, Z., de Mendoza, A., Sebé-Pedrós, A., Brown, M. W., Kramer, E., Carr, M., Kerner, P., Vervoort, M., Sánchez-Pons, N., Torruella, G., Derelle, R., Manning, G., Lang, B. F., Russ, C., Haas, B. J., Roger, A. J., Nusbaum, C., & Ruiz-Trillo, I. (2013). The *Capsaspora* genome reveals a complex unicellular prehistory of animals [Nature Publishing Group]. In *Nature Communications* (Vol. 4, Issue 1). <https://doi.org/10.1038/ncomms3325>
- Suga, H., Dacre, M., de Mendoza, A., Shalchian-Tabrizi, K., Manning, G., & Ruiz-Trillo, I. (2012).

- Genomic survey of premetazoans shows deep conservation of cytoplasmic tyrosine kinases and multiple radiations of receptor tyrosine kinases. [American Association for the Advancement of Science]. In *Science signaling* (Vol. 5, Issue 222). <https://doi.org/10.1126/scisignal.2002733>
- Suga, H., Koyanagi, M., Hoshiyama, D., Ono, K., Iwabe, N., Kuma, K. I., & Miyata, T. (1999). Extensive gene duplication in the early evolution of animals before the parazoan-eumetazoan split demonstrated by G proteins and protein tyrosine kinases from sponge and hydra. In *Journal of Molecular Evolution* (Vol. 48, Issue 6). <https://doi.org/10.1007/PL00006508>
- Suga, H., Kuma, K. I., Iwabe, N., Nikoh, N., Ono, K., Koyanagi, M., Hoshiyama, D., & Miyata, T. (1997). Intermittent divergence of the protein tyrosine kinase family during animal evolution. In *FEBS Letters* (Vol. 412, Issue 3). [https://doi.org/10.1016/S0014-5793\(97\)00639-X](https://doi.org/10.1016/S0014-5793(97)00639-X)
- Suga, H., & Ruiz-Trillo, I. (2013). Development of ichthyosporeans sheds light on the origin of metazoan multicellularity. *Developmental Biology*, 377(1), 284–292. <https://doi.org/10.1016/j.ydbio.2013.01.009>
- Suga, H., Sasaki, G., Kuma, K. ichi, Nishiyori, H., Hirose, N., Su, Z. H., Iwabe, N., & Miyata, T. (2008). Ancient divergence of animal protein tyrosine kinase genes demonstrated by a gene family tree including choanoflagellate genes. In *FEBS Letters* (Vol. 582, Issue 5). <https://doi.org/10.1016/j.febslet.2008.02.002>
- Suga, H., Torruella, G., Burger, G., Brown, M. W., & Ruiz-Trillo, I. (2014). Earliest holozoan expansion of phosphotyrosine signaling. In *Molecular Biology and Evolution* (Vol. 31, Issue 3). <https://doi.org/10.1093/molbev/mst241>
- Svedberg, J., Hosseini, S., Chen, J., Vogan, A. A., Mozgova, I., Hennig, L., Manitchotpisit, P., Abusharekh, A., Hammond, T. M., Lascoux, M., & Johannesson, H. (2018). Convergent evolution of complex genomic rearrangements in two fungal meiotic drive elements [Nature Publishing Group]. In *Nature Communications* (Vol. 9, Issue 1). <https://doi.org/10.1038/s41467-018-06562-x>
- Szathmáry, E. (2015). Toward major evolutionary transitions theory 2.0. *Proceedings of the National Academy of Sciences of the United States of America*, 112(33), 10104–10111. <https://doi.org/10.1073/pnas.1421398112>
- Szathmáry, E., & Smith, J. M. (1995). The major evolutionary transitions. *Nature*, 374(6519), 227–232. <https://doi.org/10.1038/374227a0>
- Takeshita, N. (2016). Coordinated process of polarized growth in filamentous fungi [Taylor & Francis]. In *Bioscience, Biotechnology, and Biochemistry* (Vol. 80, Issue 9). <https://doi.org/10.1080/09168451.2016.1179092>
- Takeshita, N., Higashitsuji, Y., Konzack, S., & Fischer, R. (2008). Apical Sterol-rich Membranes Are Essential for Localizing Cell End Markers That Determine Growth Directionality in the Filamentous Fungus *Aspergillus nidulans*. *Molecular Biology of the Cell*, 19(1), 339–351. <https://doi.org/10.1091/mbc.e07-06-0523>
- Takeshita, N., Yamashita, S., Ohta, A., & Horiuchi, H. (2006). *Aspergillus nidulans* class V and VI chitin synthases CsmA and CsmB, each with a myosin motor-like domain, perform compensatory functions that are essential for hyphal tip growth. *Molecular Microbiology*, 59(5), 1380–1394. <https://doi.org/10.1111/j.1365-2958.2006.05030.x>
- Tanabe, Y., Saikawa, M., Watanabe, M. M., & Sugiyama, J. (2004). Molecular phylogeny of Zygomycota based on EF-1 α and RPB1 sequences: Limitations and utility of alternative markers to rDNA. In *Molecular Phylogenetics and Evolution* (Vol. 30, Issue 2). [https://doi.org/10.1016/S1055-7903\(03\)00185-4](https://doi.org/10.1016/S1055-7903(03)00185-4)
- Taylor T. N., Krings M., Taylor E.L. (2014). Fossil fungi. San Diego, CA, USA: Academic Press.
- Taylor, L. L., Leake, J. R., Quirk, J., Hardy, K., Banwart, S. A., & Beerling, D. J. (2009a). Biological

- weathering and the long-term carbon cycle: Integrating mycorrhizal evolution and function into the current paradigm. *Geobiology*, 7(2), 171–191. <https://doi.org/10.1111/j.1472-4669.2009.00194.x>
- Taylor, T. N., E.L. Taylor, and M. Krings. (2009b). *Paleobotany: The Biology and Evolution of Fossil Plants*. Academic Press, New York, NY. 1230 pp.
- Taylor, T. N., Hass, H., Kerp, H., Krings, M., & Hanlin, R. T. (2005). Perithecial ascomycetes from the 400 million year old Rhynie chert: an example of ancestral polymorphism. *Mycologia*, 97(1), 269–285.
- Taylor, T. N., Remy, W., & Hass, H. (1992). Parasitism in a 400-million-year-old green alga. *Nature*, 357(6378), 493–494. <https://doi.org/10.1038/357493a0>
- Tebarth, B., Doedt, T., Krishnamurthy, S., Weide, M., Monterola, F., Dominguez, A., & Ernst, J. F. (2003). Adaptation of the Efg1p Morphogenetic Pathway in *Candida albicans* by Negative Autoregulation and PKA-dependent Repression of the EFG1 Gene [Academic Press]. In *Journal of Molecular Biology* (Vol. 329, Issue 5). [https://doi.org/10.1016/S0022-2836\(03\)00505-9](https://doi.org/10.1016/S0022-2836(03)00505-9)
- Torruella, G., Grau-Bové, X., Moreira, D., Karpov, S. A., Burns, J. A., Sebé-Pedrós, A., Völcker, E., & López-García, P. (2018). Global transcriptome analysis of the aphelid *Paraphelidium tribonemae* supports the phagotrophic origin of fungi [Nature Publishing Group]. In *Communications Biology* (Vol. 1, Issue 1). <https://doi.org/10.1038/s42003-018-0235-z>
- Umen, J. G. (2014). Green Algae and the Origins of Multicellularity in the Plant Kingdom [Cold Spring Harbor Laboratory Press]. In *Cold Spring Harbor Perspectives in Biology* (Vol. 6, Issue 11). <https://doi.org/10.1101/cshperspect.a016170>
- van Dongen, S. (2000). Graph clustering by flow simulation. *Graph Stimulation by Flow Clustering, PhD thesis*, University of Utrecht. <https://doi.org/10.1016/j.cosrev.2007.05.001>
- Van Donk, E., & Lurling, M. (2000). Grazer-induced colony formation in *Scenedesmus* : are there costs to being colonial ? In *Oikos* (Vol. 88, Issue 1).
- Vargas, M. M., Aronson, J. M., & Roberson, R. W. (1993). The cytoplasmic organization of hyphal tip cells in the fungus *Allomyces macrogynus*. In *Protoplasma* (Vol. 176, Issues 1–2). <https://doi.org/10.1007/BF01378938>
- Vierula, P. J., & Mais, J. M. (1997). A gene required for nuclear migration in *Neurospora crassa* codes for a protein with cysteine-rich, LIM/RING-like domains. In *Molecular microbiology* (Vol. 24, Issue 2). <http://www.ncbi.nlm.nih.gov/pubmed/9159520>
- Virag, A., & Harris, S. D. (2006). The Spitzenkörper: a molecular perspective [Elsevier]. In *Mycological Research* (Vol. 110, Issue 1). <https://doi.org/10.1016/J.MYCRES.2005.09.005>
- Virag, A., Lee, M. P., Si, H., & Harris, S. D. (2007). Regulation of hyphal morphogenesis by *cdc42* and *rac1* homologues in *Aspergillus nidulans*. In *Molecular Microbiology* (Vol. 66, Issue 6). <https://doi.org/10.1111/j.1365-2958.2007.06021.x>
- Wagner, A. R., Luan, Q., Liu, S.-L., & Nolen, B. J. (2013). Dip1 defines a class of Arp2/3 complex activators that function without preformed actin filaments. *Current Biology : CB*, 23(20), 1990–1998. <https://doi.org/10.1016/j.cub.2013.08.029>
- Wagner, G. P., Amemiya, C., & Ruddle, F. (2003). Hox cluster duplications and the opportunity for evolutionary novelties. *Proceedings of the National Academy of Sciences*, 100(25), 14603–14606. <https://doi.org/10.1073/PNAS.2536656100>
- Walther, A., & Wendland, J. (2004). Polarized hyphal growth in *Candida albicans* requires the Wiskott-Aldrich Syndrome protein homolog Wallp. *Eukaryotic Cell*, 3(2), 471–482. <https://doi.org/10.1128/EC.3.2.471-482.2004>

- Wang, B., Yeun, L. H., Xue, J. Y., Liu, Y., Ané, J. M., & Qiu, Y. L. (2010). Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytologist*, 186(2), 514–525. <https://doi.org/10.1111/j.1469-8137.2009.03137.x>
- Wang, L., Zhai, B., & Lin, X. (2012). The Link between Morphotype Transition and Virulence in *Cryptococcus neoformans*. *PLoS Pathogens*, 8(6), e1002765. <https://doi.org/10.1371/journal.ppat.1002765>
- Ward, M. P., Gimeno, C. J., Fink, G. R., & Garrett, S. (1995). SOK2 may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. *Molecular and Cellular Biology*, 15(12), 6854–6863. <https://doi.org/10.1128/MCB.15.12.6854>
- Wendland, J. (2001). Comparison of Morphogenetic Networks of Filamentous Fungi and Yeast. *Fungal Genetics and Biology*, 34(2), 63–82. <https://doi.org/10.1006/FGBI.2001.1290>
- Wessels, J. G. H. (1986). *Cell Wall Synthesis in Apical Hyphal Growth* (G. H. B. T.-I. R. of C. Bourne (ed.); Vol. 104, pp. 37–79). Academic Press. [https://doi.org/https://doi.org/10.1016/S0074-7696\(08\)61923-3](https://doi.org/https://doi.org/10.1016/S0074-7696(08)61923-3)
- Wessels, J. G. H. (1988). A steady-state model for apical wall growth in fungi. In *Acta Botanica Neerlandica* (Vol. 37, Issue 1). <https://doi.org/10.1111/j.1438-8677.1988.tb01576.x>
- Winkelströter, L. K., Bom, V. L. P., de Castro, P. A., Ramalho, L. N. Z., Goldman, M. H. S., Brown, N. A., Rajendran, R., Ramage, G., Bovier, E., dos Reis, T. F., Savoldi, M., Hagiwara, D., & Goldman, G. H. (2015). High osmolarity glycerol response PtcB phosphatase is important for *Aspergillus fumigatus* virulence. *Molecular Microbiology*, 96(1), 42–54. <https://doi.org/10.1111/mmi.12919>
- Wolpert, L., & Szathmáry, E. (2002). Multicellularity: evolution and the egg. *Nature*, 420(6917), 745. <https://doi.org/10.1038/420745a>
- Wösten, H. A. B. (2019). Filamentous fungi for the production of enzymes, chemicals and materials. In *Current Opinion in Biotechnology* (Vol. 59). <https://doi.org/10.1016/j.copbio.2019.02.010>
- Wu, J., Liu, Y., Lv, W., Yue, X., Que, Y., Yang, N., Zhang, Z., Ma, Z., Talbot, N. J., & Wang, Z. (2015). FgRIC8 is involved in regulating vegetative growth, conidiation, deoxynivalenol production and virulence in *Fusarium graminearum* [Academic Press]. In *Fungal Genetics and Biology* (Vol. 83). <https://doi.org/10.1016/J.FGB.2015.08.012>
- Xu, X., & Jin, T. (2019). ELMO proteins transduce G protein-coupled receptor signal to control reorganization of actin cytoskeleton in chemotaxis of eukaryotic cells [Taylor & Francis]. In *Small GTPases* (Vol. 10, Issue 4). <https://doi.org/10.1080/21541248.2017.1318816>
- Xue, C., Hsueh, Y.-P., & Heitman, J. (2008). Magnificent seven: roles of G protein-coupled receptors in extracellular sensing in fungi. *FEMS Microbiology Reviews*, 32(6), 1010–1032. <https://doi.org/10.1111/j.1574-6976.2008.00131.x>
- Yamazaki, H., Tanaka, A., Kaneko, J., Ohta, A., & Horiuchi, H. (2008). *Aspergillus nidulans* ChiA is a glycosylphosphatidylinositol (GPI)-anchored chitinase specifically localized at polarized growth sites [Academic Press]. In *Fungal Genetics and Biology* (Vol. 45, Issue 6). <https://doi.org/10.1016/J.FGB.2008.02.008>
- Yang, F., Ma, D., Wan, Z., Liu, W., Ji, Y., & Li, R. (2011). The Role of sho1 in Polarized Growth of *Aspergillus fumigatus* [Springer Netherlands]. In *Mycopathologia* (Vol. 172, Issue 5). <https://doi.org/10.1007/s11046-011-9452-4>
- Young, C., Mapes, J., Hanneman, J., Al-Zarban, S., & Ota, I. (2002). Role of Ptc2 Type 2C Ser/Thr Phosphatase in Yeast High-Osmolarity Glycerol Pathway Inactivation. *Eukaryotic Cell*, 1(6), 1032–1040. <https://doi.org/10.1128/EC.1.6.1032-1040.2002>

- Zhang, J. (2003). Evolution by gene duplication: An update. In *Trends in Ecology and Evolution* (Vol. 18, Issue 6). [https://doi.org/10.1016/S0169-5347\(03\)00033-8](https://doi.org/10.1016/S0169-5347(03)00033-8)
- Zhang, J., Rosenberg, H. F., & Nei, M. (1998). Positive Darwinian selection after gene duplication in primate ribonuclease genes. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 95, Issue 7). <https://doi.org/10.1073/pnas.95.7.3708>
- Zhang, J., Zhang, Y. ping, & Rosenberg, H. F. (2002). Adaptive evolution of a duplicated pancreatic ribonuclease gene in a leaf-eating monkey. In *Nature Genetics* (Vol. 30, Issue 4). <https://doi.org/10.1038/ng852>
- Zhao, W., Li, C., Liang, J., & Sun, S. (2014). The *Aspergillus fumigatus* β -1,3-glucanosyltransferase Gel7 plays a compensatory role in maintaining cell wall integrity under stress conditions. In *Glycobiology* (Vol. 24, Issue 5). <https://doi.org/10.1093/glycob/cwu003>
- Zhao, Y., Su, H., Zhou, J., Feng, H., Zhang, K.-Q., & Yang, J. (2015). The APSES family proteins in fungi: Characterizations, evolution and functions. *Fungal Genetics and Biology*, 81, 271–280. <https://doi.org/10.1016/J.FGB.2014.12.003>
- Zou, J., Friesen, H., Larson, J., Huang, D., Cox, M., Tatchell, K., Andrews, B. (2009). Regulation of Cell Polarity through Phosphorylation of Bni4 by Pho85 G1 Cyclin-dependent Kinases in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell*, 20, 3239-3250. <https://doi.org/10.1091/mbc.e08-12-1255>

Summary

Multicellularity evolved independently over 25 times from unicellular ancestors during evolution. In contrast to animals and plants, where multicellularity can be acquired either by clonal or aggregative development, fungal multicellularity emerged through an alternative route - by forming filamentous hyphae. Although the genetic background of the morphogenetic machinery behind hyphal growth is well characterized, the evolutionary-genetic origins of hyphal multicellularity are still unknown.

In my Ph.D. project, my main goal was to investigate the evolutionary origins of hyphal multicellularity and answer our emerging questions related to this topic.

To understand the evolutionary origin of multicellular hyphal growth, a species phylogeny was constructed. The phylogeny represented 71 species, including 4 unicellular, 39 hyphal, 15 secondarily simplified fungi, as well as 13 non-fungal relatives. To infer the growth mode of fungal ancestors and the origin of hyphal multicellularity an ancestral character state reconstruction was performed across our phylogeny. According to the results, hyphal multicellularity possibly evolved at the split of Blastocladiomycota, Chytridiomycota and Zoopagomycota lineages.

In order to investigate whether those genetic innovations that were previously found to be key contributors to metazoan multicellularity are also crucial to hyphal multicellularity, comparative phylogenomic analyses were performed on Ser/Thr kinases, hybrid histidine kinases, G protein-coupled receptors and adhesion-related proteins. The analyses revealed no significant expansions of metazoan multicellularity-specific gene families in filamentous fungi. These results highlighted important differences between fungal and animal multicellularity, and show that the diversification of 'classic' metazoan multicellularity-related gene families can't explain the multicellularity in hypha-forming fungi.

Furthermore, we reconstructed the evolutionary history of 362 known hypha morphogenesis-related gene families. The results showed a mixed picture. 50% (181) of the gene families were older than hypha morphogenesis itself. Significant proportion (71%, 128) of these gene families was ancient and conserved across all eukaryotes with very few or no duplication events at the origin of hyphal multicellularity. Only a subset of these conserved gene families comprised families that have deep eukaryotic origins, but also show elevated duplication numbers coincident with the evolution of hyphae. Total 4.7 % (17) of gene families coincided with the evolution of hyphae. These gene

families could have evolved *de novo* at the origin of hyphal growth, representing genetic innovations related to the evolution of hyphal multicellularity. 45.3% (164) of the investigated gene families originated after the emergence of hyphal growth, representing lineage and species-specific genetic innovations related to the evolution of hyphae. Overall 25.7% (93) of the investigated gene families showed duplications at the origin of hyphae. These gene duplications were characteristic mainly in “cell wall biogenesis and remodeling” and “transcriptional regulation” functional categories. Separate analyses on each gene families, however revealed no families having significantly elevated duplication rate at the origin of hyphal growth. These results indicate a lack of a major burst of *de novo* gene family origin coincident with the evolution of hyphae, and also limited genetic novelty by gene duplications in regard to hyphal multicellularity.

To answer, whether changes in gene architecture (e.g. gene, intron and coding sequence length) show any correlation with hyphal multicellularity, we investigated gene structures of hypha morphogenesis-related genes. Our analyses revealed significant differences between genes of unicellular and multicellular fungi, and showed that modifications of CDS and intron length in multicellular fungi could have contributed to the evolution of hyphal multicellularity.

Although phagocytosis is thought to be absent in fungal species, our hypha morphogenesis gene set included several entries associated with this process. We therefore examined the evolutionary history of 414 phagocytosis-related gene families in multicellular fungi based on the phagocytotic machinery of other eukaryotes. The analysis of phagocytotic gene families revealed, that despite the loss of phagocytosis itself, several phagocytotic genes are conserved in fungal species. These findings suggest, that the ancient eukaryotic toolkit responsible for phagocytosis possibly adapted during evolution for serving other purposes in hyphal growth and multicellularity.

A systematic genome-wide search was also performed for additional hyphal multicellularity-related gene families, based on the criteria that hyphal multicellularity-related genes should originate or diversify at the origin of hyphae and be conserved in most filamentous fungi. Our search predicted a link to the evolution of hyphal multicellularity for another 414 gene families. Also, we found that out of these 414 families, 114 originated at the emergence of hyphal growth. These gene families consist of already known hyphal morphogenesis-related gene families, but also uncharacterized ones too, having no information about their functions and roles in hyphal growth. These

newly identified, unknown gene families can serve potential candidates for further studies on hyphal morphogenesis.

Additionally, we investigated gene loss events across the evolution of five yeast-like lineages to reveal, whether the severe morphological reduction, that is specific to yeast-like fungi was associated with genomic changes. This analysis highlighted that 54-65% of hyphal morphogenesis-related genes were retained in yeasts species, suggesting that in general, big part of the morphogenetic toolkit responsible for hyphal growth is less dispensable for yeasts, than are other genes with other functions. The finding that the genomes of yeast-like fungi have a relatively big proportion of the morphogenetic toolkit responsible for hyphal growth can answer the question, that how yeast-like fungi are capable to produce elongated filaments, pseudohyphae and true hyphae in response to different kinds of external signals.

In conclusion, our results show that the most important driving forces of the evolution of hyphal multicellularity may have been the extensive co-option and exaptation of ancient eukaryotic morphogenetic toolkits. These processes were probably accompanied by gene duplication events and limited *de novo* gene family birth at the origin of hyphal growth, and changes in gene structures. Our findings related to hyphal multicellularity in fungi support the view coming from previous investigations of other multicellular organisms such as animals and plants, that in terms of genetic innovations, transitions to multicellular life represent a minor rather a major evolutionary step. Also, our observations suggest that the prerequisites for the evolution of hyphal multicellularity differs considerably from that of animals. These differences raise the possibility that in addition to the aggregative and clonal modes of evolving multicellularity, hyphal multicellularity should be considered a third, qualitatively different route to multicellular development.

Összefoglaló

A soksejtűség az evolúció során több, mint 25 alkalommal, egymástól függetlenül alakult ki a különböző élőlénycsoportokban egysejtű közös ősből. Ellentétben az állatokkal és növényekkel, ahol a többsejtűség klonális vagy aggregatív fejlődéssel jött létre, a gombák a soksejtűség egy alternatív útját választották, azáltal, hogy gombákra specifikus soksejtű képleteket, úgynevezett hifákat hoztak létre. Bár a hifás növekedés mögött álló mechanizmus genetikai háttere jól ismert, a hifás soksejtűség evolúciós-genetikai eredete még mindig ismeretlen a számunkra.

Doktori tanulmányaim során a fő célom a hifás soksejtűség evolúciós eredetének vizsgálata volt.

A többsejtű hifás növekedés evolúciós eredetének megértése céljából filogenetikai törzsfát rekonstruáltunk. A törzsfá 71 faj, köztük 4 egysejtű, 39 hifás és 15 másodlagosan leegyszerűsödött, élesztőszerű gomba, valamint 13 nem gomba rokon faj leszármazását reprezentálta. A gomba ősök karakterállapotának és a hifás soksejtűség eredetének megbecsüléséhez ősi karakterállapot rekonstrukciót hajtottunk végre. Eredményeink szerint a hifás soksejtűség nagy valószínűséggel a Blastocladiomycota, a Chytridiomycota és a Zoopagomycota törzsek szétválásakor alakult ki a gombák evolúciója során.

Annak érdekében, hogy feltérképezzük az állati soksejtűségben kulcs szerepet játszó genetikai újítások esetleges fontosságát a hifás soksejtűség evolúciójában, összehasonlító genomikai analízist végeztünk Ser/Thr kinázokat, hibrid hisztidin kinázokat, G fehérje kapcsolt receptorokat és adhézióban szerepet játszó fehérjéket kódoló géncsaládokon. Analízisünk azonban nem mutatta ki ezeknek az állati soksejtűségben fontos szerepet játszó géncsaládoknak a szignifikáns expanzióját soksejtű gombákban. Eredményeink rávilágítottak a gombák és az állatok soksejtűsége közötti fontos különbségekre, illetve arra, hogy a „klasszikus”, állati soksejtűséggel kapcsolatos géncsaládok diverzifikációja nem magyarázhatja a hifaképző gombák soksejtűségét.

A klasszikus, soksejtűségben szerepet játszó géncsaládok vizsgálata mellett rekonstruáltuk 362 ismert, hifa morfogenezisben szerepet játszó géncsalád evolúcióját. Az eredmények összetett képet mutattak. A géncsaládok 50% -a (181) idősebb volt, mint maga a hifa morfogenezis. Ezeknek az ősi géncsaládoknak a jelentős része (71%, 128) konzervációt mutatott az összes vizsgált eukariótában, és keveset vagy egyáltalán nem

mutatott duplikációt a hifás növekedés megjelenésének feltételezett időpontjában. A géncsaládok összesen 4,7%-a (17) mutatott összefüggést a hifás növekedés evolúciójával. Ennek a 17 géncsaládnak a *de novo* születése egybeesett a hifás növekedés megjelenésével, olyan genetikai újításokat reprezentálva ezáltal, melyek összefüggésbe hozhatók a hifás soksejtűség evolúciójával. A vizsgált géncsaládok 45,3%-a (164) a hifás soksejtűség megjelenése után keletkezett, mely a hifás növekedés evolúciójával kapcsolatos fajspecifikus genetikai újításokat képviseli. Összességében az analízisben vizsgált géncsaládok 25.7%-a (93) mutatott génduplikációs eseményt a hifa megjelenésének feltételezett időpontjában. Ezek a duplikációk főként a sejtfal biogenezisben és transzkripciós szabályozásban szerepet játszó géncsaládokra voltak jellemzőek. Az egyes géncsaládokon elvégzett statisztikai elemzések azonban nem tártak fel szignifikánsan megemelkedett duplikációs rátát, mely összefüggésbe hozható a hifás növekedés evolúciójával. Ezekből az eredményekből arra következtethetünk, hogy a hifás növekedés megjelenésével nem hozható összefüggésbe robbanásszerű *de novo* géncsalád születés és géncsalád diverzifikáció.

Annak megválaszolására, hogy a génszerkezeti változások (pl. gén, intron vagy kódoló szekvencia hossz változás) mutatnak-e összefüggést a hifás soksejtűséggel, vizsgáltuk a hifa morfogenezisben szerepet játszó gének génszerkezeti tulajdonságait egysejtű és soksejtű gombákban. Elemzéseink jelentős különbségeket tártak fel az egysejtű és a többsejtű gombák génjei között, valamint megmutatták, hogy a kódoló szekvencia hossz és az intron hossz módosulása a többsejtű gombákban hozzájárulhatott a hifás soksejtűség kialakulásához.

Habár a fagocitózis képessége elveszett gombákban, mégis számos olyan gént tartalmazott a hifa morfogenezis génadatbázisunk, mely kapcsolatba hozható más eukarióták fagocitotikus folyamataival. Ezért más eukarióták fagocitotikus eszköztára alapján 414 fagocitózisban szerepet játszó géncsalád evolúcióját vizsgáltuk soksejtű gombákban. Ezeknek a fagocitotikus géncsaládoknak az elemzése feltárta, hogy a fagocitózis képességének elvesztése ellenére számos fagocitotikus gén konzerválódott soksejtű gombákban. Ezek a megállapítások azt sugallják, hogy a fagocitózisért felelős ősi eukarióta eszköztár a gombák evolúciója során esetleg más, hifa morfogenezishez köthető folyamatokhoz adaptálódott.

Szisztematikus genomszintű keresést végeztünk annak megválaszolására, hogy léteznek-e további olyan géncsaládok, melyek evolúciós eredete és dinamikája összefüggésbe hozható a hifás soksejtűség megjelenésével. Keresésünk 414 géncsalád

esetében talált összefüggést a hifa evolúciójával, melyből 114 géncsalád megjelenése egybeesett a hifás soksejtűség megjelenésével. Az azonosított géncsaládok tartalmaztak már ismert, hifa morfogenezissel kapcsolatos géncsaládokat, de ezidáig ismeretlen, funkcionális annotáció nélküli géncsaládokat is. Ezek az újonnan azonosított, ismeretlen géncsaládok potenciális jelöltként szolgálhatnak a hifás soksejtűség további vizsgálataihoz.

Az élesztőszerű gombák hifaképző képessége erősen lecsökkent. Annak a kérdésnek a megválaszolására, hogy ez az élesztőszerű gombákra jellemző morfológiai egyszerűsödés összefügg-e genom szintű változásokkal, vizsgáltuk a génvesztéses eseményeket öt élesztőszerű törzs evolúciója során. Analízisünk szerint a hifa morfogenezisben szerepet játszó gének 54-65%-a megtalálható az élesztő fajokban. Mindez arra utal, hogy a hifás növekedésért felelős genetikai eszköztár kevésbé nélkülözhető, mint más funkciójú gének, és magyarázhatja azt, hogy a nagyfokú morfológiai redukció ellenére az élesztőszerű gombák mégis képesek pszeudohifák, illetve valódi hifák képzésére, mely egyfajta válaszreakció a változó környezeti ingerekre.

Eredményeink azt mutatják, hogy a hifás soksejtűség evolúciójának legfontosabb mozgatórugója az ősi eukarióta morfogenetikus eszköztár kiterjedt kooperációja és exaptációja volt. Ezt a folyamatot nagy valószínűséggel kis fokú géncsalád diverzifikáció és korlátozott számú *de novo* géncsalád-születés kísérte. A gombák hifás soksejtűségével kapcsolatos megállapításaink alátámasztják más soksejtű élőlények, például az állatok és növények korábbi vizsgálataiból származó feltételezéseket, miszerint a soksejtűvé válást, mint nagy fenotípusos változást viszonylag kis számú genetikai innováció kíséri. Továbbá megfigyeléseink alátámasztják, hogy a hifás soksejtűség kialakulásának előfeltételei jelentősen eltérnek az állatokétól. Ezek a különbségek felvetik annak lehetőségét, hogy a soksejtűvé válás aggregatív és klonális módjai mellett a hifás soksejtűséget egy harmadik, minőségileg eltérő útnak kell tekinteni.

List of publications (MTMT ID: 10055083)

Mandatory peer-reviewed international publication on which this thesis is based:

Kiss, E., Hegedüs, B., Virágh, M., Varga, T., Merényi, Z., Kószó, T., Bálint, B., Prasanna, AN., Krizsán, K., Kocsubé, S., Riquelme, M., Takeshita, N., Nagy, GL, “Comparative genomics reveals the origin of fungal hyphae and multicellularity”, *Nature Communications* 2019 Sep 9;10(1):4080

Mandatory peer-reviewed international publication for the fulfillment of doctoral process:

Miyauchi, S., Kiss, E., Kuo, A., Drula, E., Kohler, A., Sánchez-García, M., Andreopoulos, B., Barry, K.W., Bonito, G., Buée, M., Carver, A., Chen, C., Cichocki, N., Clum, A., Culley, D., Crous, P.W., Fauchery, L., Girlanda, M., Hayes, R.D., Kéri, Z., LaButti, K., Lipzen, A., Lombard, V., Magnuson, J., Maillard, F., Morin, E., Murat, C., Nolan, M., Ohm, R. A., Pangilinan, J., de Freitas Pereira, M., Perotto, S., Peter, M., Pfister, S., Riley, R., Sitrit, Y., Stielow, B.J., Szöllősi, G., Žifčáková, L., Štursová, M., Spatafora, J.W., Tedersoo, L., Vaario, L.M., Yamada, A., Yan, M., Wang, P., Xu, J., Bruns, T., Baldrian, P., Vilgalys, R., Dunand, C., Henrissat, B., Grigoriev, I.V., Hibbett, D., Nagy, GL., Martin FM, “Large scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits”, *Nature Communications* 2020

Other peer-reviewed international publications:

Nagy, GL., Tóth, R., Kiss, E., Slot, J., Gácsér, A., Kovács, GM, ”Six Key Traits of Fungi: Their Evolutionary Origins and Genetic Bases”, *Microbiol Spectrum* 5(4):FUNK-0036-2016.

Book Chapter:

Nagy, GL., Tóth, R., Kiss, E., Slot, J., Gácsér, A., Kovács, GM, ”Six Key Traits of Fungi: Their Evolutionary Origins and Genetic Bases” In: Heitman J, Howlett BJ, Crous PW, Stukebrock EH, James TY, Gow NAR (szerk.) *The Fungal Kingdom*. New York: American Society for Microbiology Press (ASM), 2017. pp. 35-56. (ISBN: 9781555819576)

Appendix

1. List of 71 genomes used in comparative genomic analysis.

[illegible]

UC=pleiomorphically unicellular fungi, HMC=hyphal multicellular fungi, YE=yeast like, secondarily unicellular, NF=non-fungal unicellular protist, NFM=non-fungal multicellular)

2. Phylogenetic age distribution of hypha morphogenesis genes. Mirrors Figure 27. with gene names provided for each dot.

3. List of gene families showing duplication events at the time of the emergence of filamentous fungi (BCZ nodes), based on COMPARE analysis.

number of duplications in BCZ nodes	functional category	cluster number	<i>A.fumigatus</i> ortholog	<i>S. cerevisiae</i> ortholog	ortholog from other species
2	actin cytoskeleton	5953	Afu8g04520	<i>SLA1/YBL007C</i>	
1	actin cytoskeleton	1561	Afu6g07910	<i>SLM1/YIL105C</i>	
1	actin cytoskeleton	2105	NA	NA	<i>WALI/CR_09650W (C.albicans)</i>
1	actin cytoskeleton	4346	NA	<i>END3/YNL084C</i>	
1	actin cytoskeleton	4903	Afu7g03870	<i>PAN1/YIR006C</i>	
1	actin cytoskeleton	13227	NA	<i>VRP1/YLR337C</i>	
1	actin cytoskeleton	17934	NA	<i>EDE1/YBL047C</i>	
1	adhesion	417	<i>GLFB/Afu3g12700</i>	<i>YMD8/YML038C</i>	
1	adhesion	4291	NA	NA	<i>IFF4/CR_00610W (C.albicans)</i>
1	adhesion	7971	Afu1g01540	NA	
3	cell cycle regulation	200	Afu4g12160	<i>CLB2/YPR119W</i>	
2	cell cycle regulation	2915	Afu4g06130	<i>WHI2/YOR043W</i>	
8	cell wall biogenesis	234	<i>CHSE/Afu2g13440</i>	NA	
6	cell wall biogenesis	368	<i>CHSF/Afu8g05630</i>	<i>CHS3/YBR023C</i>	
6	cell wall biogenesis	374	<i>CRH3/Afu3g09250</i>	<i>UTR2/YEL040W</i>	
5	cell wall biogenesis	209	<i>CHS4/Afu2g01870</i>	<i>CHS1/YNL192W</i>	
4	cell wall biogenesis	435	<i>GEL7/Afu6g12410</i>	<i>GAS1/YMR307W</i>	
4	cell wall biogenesis	635	<i>FKS1/Afu6g12400</i>	<i>FKS1/YLR342W</i>	
4	cell wall biogenesis	636	<i>CHL1/Afu5g03760</i>	<i>CTS1/YLR286C</i>	
3	cell wall biogenesis	171	<i>EXG1/Afu1g03600</i>	<i>EXG1/YLR300W</i>	
3	cell wall biogenesis	3398	Afu8g02320	NA	
2	cell wall biogenesis	222	<i>ORLA/Afu3g05650</i>	<i>TPS3/YMR261C</i>	
2	cell wall biogenesis	293	<i>KRE6/Afu2g11870</i>	<i>KRE6/YPR159W</i>	
2	cell wall biogenesis	361	<i>CHS3/Afu8g05620</i>	<i>SKT5/YBL061C</i>	
1	cell wall biogenesis	166	<i>PMT4/Afu8g04500</i>	<i>PMT1/YDL095W</i>	
1	cell wall biogenesis	843	Afu4g00620	<i>DFG5/YMR238W</i>	
1	cell wall biogenesis	4470	<i>SUN1/Afu7g05450</i>	<i>SUN4/YNL066W</i>	
1	cell wall biogenesis	9065	<i>CHSD/Afu1g12600</i>	NA	
6	microtubular transport	7	Afu6g12970	<i>PAC1/YOR269W</i>	
3	microtubular transport	40	Afu6g14220	<i>KIP1/YBL063W</i>	
3	microtubular transport	4601	NA	NA	<i>KIN4/XM_400772 (U.maydis)</i>
2	microtubular transport	1015	<i>EB1/Afu3g11860</i>	<i>BIM1/YER016W</i>	
1	microtubular transport	82	Afu1g13390	<i>TUB3/YML124C</i>	
1	microtubular transport	96	Afu5g11810	<i>DYN1/YKR054C</i>	
7	polarity establishment	47	<i>RACA/Afu3g06300</i>	<i>RHO4/YKR055W</i>	
4	polarity establishment	62	Afu6g06870	<i>YCK2/YNL154C</i>	
3	polarity establishment	2482	Afu4g04120	<i>BEM1/YBR200W</i>	
2	polarity establishment	52	<i>RASA/Afu5g11230</i>	<i>RSR1/YGR152C</i>	
2	polarity establishment	484	<i>ROM2/Afu5g08550</i>	<i>ROM1/YGR070W</i>	
2	polarity establishment	2900	<i>RGSB/Afu4g12640</i>	<i>RAX1/YOR301W</i>	
2	polarity establishment	3689	Afu6g04940	<i>BNR1/YIL159W</i>	
1	polarity establishment	336	Afu4g06290	<i>LGI1/YHL003C</i>	
1	polarity establishment	492	Afu3g06080	<i>MSS4/YDR208W</i>	
1	polarity establishment	1214	Afu2g15050	<i>LRG1/YDL240W</i>	
1	polarity establishment	2379	Afu8g02810	NA	
1	polarity establishment	8082	Afu2g08180	NA	
1	polarity establishment	14418	NA	<i>BOI1/YBL085W</i>	
1	polarity establishment	30051	NA	NA	<i>TE44/SPBC1706.01 (S.pombe)</i>
5	septation	25	Afu5g02220	<i>KIC1/YHR102W</i>	
2	septation	87	<i>ASP4/Afu5g08540</i>	<i>CDC11/YJR076C</i>	
2	septation	3476	Afu6g11010	<i>TAO3/YIL129C</i>	
1	septation	3097	NA	NA	<i>PLO1/SPAC23C11.16 (S.pombe)</i>
1	septation	3480	<i>ENGL1/Afu1g04260</i>	<i>DSE4/ENGL1/YNR067C</i>	
1	septation	7847	Afu1g13170	NA	NA
1	septation	8985	Afu5g03400	NA	<i>CYK3/NCU04095T1 (N.crassa)</i>
7	signaling	9	Afu6g02300	<i>SKS1/YPL026C</i>	
7	signaling	49	Afu5g01510	<i>SSY1/YDR160W</i>	
4	signaling	132	<i>PTCG/Afu5g13340</i>	<i>PTC1/YDL006W</i>	
3	signaling	277	<i>MEAA/Afu2g05880</i>	<i>MEP2/YNL142W</i>	
3	signaling	359	<i>ARTA/Afu2g03290</i>	<i>BMH1/YER177W</i>	
2	signaling	53	<i>SAKA/Afu1g12940</i>	<i>KSS1/YGR040W</i>	
2	signaling	1726	NA	<i>SSK2/YNR031C</i>	
2	signaling	1854	<i>SHO1/Afu5g08420</i>	<i>SHO1/YER118C</i>	
1	signaling	37	<i>SITA/Afu6g11470</i>	<i>SIT4/YDL047W</i>	
1	signaling	84	<i>GPA4/Afu1g13140</i>	<i>GPA2/YER020W</i>	
1	signaling	360	Afu4g06400	<i>RIM20/YOR275C</i>	
1	signaling	385	Afu5g06420	<i>STE11/YLR362W</i>	
1	signaling	557	<i>PKAR/Afu3g10000</i>	<i>BCY1/YIL033C</i>	
1	signaling	1037	<i>ACYA/Afu6g08520</i>	<i>CYR1/YJL005W</i>	
1	signaling	3665	NA	NA	<i>SSK1/C1_13930W (C.albicans)</i>
1	signaling	5950	<i>RIC1/Afu4g08820</i>	NA	
1	signaling	11811	NA	<i>SLN1/YIL147C</i>	
1	signaling	13039	NA	<i>NPR3/YHL023C</i>	
4	transcriptional regulation	436	Afu6g04490	<i>ROF1/YHR177W</i>	
4	transcriptional regulation	637	<i>MAT1-2/Afu3g06170</i>	<i>ROX1/YPR065W</i>	
4	transcriptional regulation	687	Afu8g05570	<i>SIN3/YOL004W</i>	
3	transcriptional regulation	2479	<i>STUA/Afu2g07900</i>	<i>PHD1/YKL043W</i>	
1	transcriptional regulation	5116	NA	NA	<i>SW16/NCU07587T0 (N.crassa)</i>
1	transcriptional regulation	8521	<i>MEA4/Afu2g13260</i>	NA	
1	transcriptional regulation	9527	<i>PTAB/Afu2g12910</i>	NA	
1	transcriptional regulation	13047	NA	<i>SKN7/YHR206W</i>	
1	transcriptional regulation	18401	NA	NA	<i>TEC1/C3_04530C (C.albicans)</i>

1	transcriptional regulation	20209	NA	SIP4/YJL089W	
1	transcriptional regulation	28159	NA	NA	RIM101/C1_14340C (<i>C.albicans</i>)
3	vesicle transport	81	Afu6g02070	DNF1/YER166W	
2	vesicle transport	27	Afu3g12080	ARF1/YDL192W	
2	vesicle transport	275	Afu5g02360	VPS1/YKR001C	
2	vesicle transport	1523	NA	NA	GEA2/C3_01390C (<i>C.albicans</i>)
1	vesicle transport	6	Afu4g08040	SEC4/YFL005W	
1	vesicle transport	137	Afu5g05550	MYO2/YOR326W	
1	vesicle transport	667	Afu6g02920	SNC1/YAL030W	
1	vesicle transport	3044	NA	NA	DDI1/C1_14420W (<i>C.albicans</i>)
1	vesicle transport	3074	Afu6g04840	NA	

4. Analysis of basic genomic features of unicellular and multicellular fungi.

Welch's t-test p-values with yellow highlight: significant difference was observed between multicellular and unicellular species in gene/CDS/intron length).

actin cytoskeleton regulation				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	2167.328864	2080.517483	0.478962747	0.7094886
average of CDS length	1712.650631	1485.006993	0.015526283	2.442433
average of intron length	454.6782334	595.5104895	0.003145312	-2.997291
adhesion				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	964.1334586	1334.27027	0.0025665646	-3.202598
average of CDS length	721.7067669	1089.891892	0.0003148798	-3.915922
average of intron length	242.4266917	244.3783784	0.9760633412	-0.03019152
cell wall biogenesis and remodeling				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	2535.021452	2462.526316	0.51334381	0.6546859
average of CDS length	2139.310231	1986.431579	0.11171432	1.59676
average of intron length	395.7112211	476.0947368	0.03956141	-2.071369
cell cycle regulation				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	1441.205479	1640.888889	0.1970098	-1.320813
average of CDS length	1148.365297	1351.185185	0.1332079	-1.546164
average of intron length	292.8401826	289.7037037	0.9512694	0.06155405
microtubular transport				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	2903.209557	3709.53937	0.002858936	-3.008728
average of CDS length	2253.880538	3138.65748	0.0005930190	-3.475414
average of intron length	649.3290194	570.8818898	0.1185406	1.562228
polarity establishment and maintenance				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	2056.170488	1835.901961	0.0404716589	2.062922
average of CDS length	1685.646091	1355.137255	0.0001178339	3.924958
average of intron length	370.5243974	480.7647059	0.0035880374	-2.952786
septation				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	1978.247033	1740.782407	0.044387008	2.018187
average of CDS length	1543.35383	1222.523148	0.001716928	3.159296
average of intron length	434.8932039	518.2592593	0.007616188	-2.68574
signaling				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	1853.531152	1919.556818	0.3665905	-0.9042651
average of CDS length	1444.248584	1484.19697	0.5451871	-0.6056936
average of intron length	409.2825677	435.3598485	0.3061139	-1.025063
transcriptional regulation				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	1707.825444	1855.702128	0.4806781	-0.7102765
average of CDS length	1471.572485	1599.787234	0.4656264	-0.7349099
average of intron length	236.2529586	255.9148936	0.6807366	-0.4137698
vesicle transport				
	multicellular fungi	unicellular fungi	p-value	t-value
average of gene length	2113.056966	2084.697183	0.7339321	0.3400815
average of CDS length	1672.487013	1537.483568	0.05088538	1.956834
average of intron length	440.5699528	547.213615	0.00003030084	-4.20975

5. Phagocytosis genes in *Dictyostelium discoideum*

Gene name	UniProt ID	Function	Citation
<i>TALA</i> talinA	UniProtKB - P0CE94 (TALA_DICDI)	recognition, mediate F-actin interaction with the membrane	Niewöhner J, Weber I, Maniak M, Müller-Taubenberger A, Gerisch G. Talin-Null Cells of Dictyostelium Are Strongly Defective in Adhesion to Particle and Substrate Surfaces and Slightly Impaired in Cytokinesis. The Journal of Cell Biology. 1997;138(2):349-361.
<i>TALB</i> talinB	UniProtKB - Q54K81 (TALB_DICDI)	recognition, Actin-binding protein required for multicellular morphogenesis. Substrate of pkgB and/or pkbA.	Niewöhner J, Weber I, Maniak M, Müller-Taubenberger A, Gerisch G. Talin-Null Cells of Dictyostelium Are Strongly Defective in Adhesion to Particle and Substrate Surfaces and Slightly Impaired in Cytokinesis. The Journal of Cell Biology. 1997;138(2):349-361.
<i>DSCA</i> Discoidin-1 subunit A	UniProtKB - P02886 (DIS1A_DICDI)	recognition, Galactose- and N-acetylgalactosamine-binding lectin. May play a role in cell-substratum adhesion rather than in cell-cell adhesion. May be necessary for the maintenance of normal elongate morphology during aggregation.	Discoidin I from Dictyostelium discoideum and Interactions with Oligosaccharides: Specificity, Affinity, Crystal Structures, and Comparison with Discoidin II Sophie V.Mathieu et al, doi: 10.1016/j.jmb.2010.05.042
<i>DSCB/C</i> Discoidin-1 subunit B/C	UniProtKB - P02887 (DIS1B_DICDI)	recognition, Galactose- and N-acetylgalactosamine-binding lectin. May play a role in cell-substratum adhesion rather than in cell-cell adhesion. May be necessary for the maintenance of normal elongate morphology during aggregation.	Discoidin I from Dictyostelium discoideum and Interactions with Oligosaccharides: Specificity, Affinity, Crystal Structures, and Comparison with Discoidin II Sophie V.Mathieu et al, doi: 10.1016/j.jmb.2010.05.043
<i>DSCD</i> Discoidin-1 subunit D	UniProtKB - P02888 (DIS1D_DICDI)	recognition, Galactose- and N-acetylgalactosamine-binding lectin. May play a role in cell-substratum adhesion rather than in cell-cell adhesion. May be necessary for the maintenance of normal elongate morphology during aggregation.	Discoidin I from Dictyostelium discoideum and Interactions with Oligosaccharides: Specificity, Affinity, Crystal Structures, and Comparison with Discoidin II Sophie V.Mathieu et al, doi: 10.1016/j.jmb.2010.05.044
<i>PHG1A</i> Putative phagocytic receptor 1a	UniProtKB - Q55FP0 (PHG1A_DICDI)	recognition, Involved in adhesion, phagocytosis of hydrophilic particles and intracellular killing of bacteria. Associates with proteins harboring glycine-rich transmembrane domains and ensures their efficient localization to the cell surface.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>PHG1B</i> Putative phagocytic receptor 1b	UniProtKB - Q54ZW0 (PHG1B_DICDI)	recognition, Involved in adhesion and phagocytosis of hydrophilic particles.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>PHG1C</i> Putative phagocytic receptor 1c	UniProtKB - Q7YTA6 (PHG1C_DICDI)	recognition	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>PHG2</i> Serine/threonine-protein kinase phg2	UniProtKB - Q54QQ1 (PHG2_DICDI)	recognition, Serine/threonine kinase regulating cell substrate adhesion, phagocytosis, motility, chemotaxis, and actin cytoskeleton organization. It is a downstream component of Rap1-regulated adhesion, and it is a regulator of myosin II phosphorylation. It is also a negative regulator of development initiation.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>COMA</i> comitin	UniProtKB - Q03380 (COMI_DICDI)	actin cytoskeleton regulator, May have a role in cell motility. It has high affinity for both G-actin and F-actin. Binds to vesicle membranes via mannose residues and, by way of its interaction with actin, links these membranes to the cytoskeleton. Comitin is an F-actin binding and membrane-associated protein from Dictyostelium discoideum, which is present on Golgi and vesicle membranes and changes its localization in response to agents affecting the cytoskeleton.	Loss of the F-Actin Binding and Vesicle-Associated Protein Comitin Leads to a Phagocytosis Defect, Schreiner et al, Eukaryot Cell. 2002 Dec; 1(6): 906-914., doi: 10.1128/EC.1.6.906-914.2002
<i>PONA</i> ponticulin	UniProtKB - P54660 (PONA_DICDI)	actin cytoskeleton regulator, Binds F-actin and nucleates actin assembly. Major high affinity link between the plasma membrane and the cortical actin network.	Phagocytosis and Macropinocytosis in Dictyostelium: Phosphoinositide-Based Processes, Biochemically Distinct, Cardelli et al, Traffic. 2001 May;2(5):311-20., 10.1034/j.1600-0854.2001.002005311.x
<i>AIP1</i> Actin-interacting protein 1	UniProtKB - P54686 (AIP1_DICDI)	actin cytoskeleton regulator, Implicated in both actin filament depolymerization and polymerization. May enhance chemotaxis by promoting cofilin-dependent actin assembly at cell leading edges. Localizes to dynamic regions of the cell cortex that are enriched in filamentous actin and rapidly redistributes to newly formed cortical protrusions, disruption: Impairment of growth, macropinocytosis, phagocytosis and movement. Prolonged cytokinesis with exaggerated cortical protrusions and production of multinucleate cells.	Phagocytosis and Macropinocytosis in Dictyostelium: Phosphoinositide-Based Processes, Biochemically Distinct, Cardelli et al, Traffic. 2001 May;2(5):311-20., 10.1034/j.1600-0854.2001.002005311.x
<i>SCRA</i> scar	UniProtKB - Q54NF8 (SCAR_DICDI)	actin cytoskeleton regulator, Involved in regulation of actin and microtubule organization. Regulates phagocytosis and macropinocytosis. Located at the tips of filopodia and at the tips of pseudopodia.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORH</i> formin	UniProtKB - Q54N00 (FORH_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments. Important for cell migration and formation, elongation and maintenance of filopodia. Specifically controls filopodial dynamics by regulating actin turnover at the barbed ends of actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORC</i> formin	UniProtKB - Q54KF1 (FORC_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORA</i> formin	UniProtKB - Q54WH2 (FORA_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORF</i> formin	UniProtKB - Q5TJ56 (FORF_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORG</i> formin	UniProtKB - Q1ZXK2 (FORG_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORE</i> formin	UniProtKB - Q5TJ57 (FORE_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORB</i> formin	UniProtKB - Q54SP2 (FORB_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORD</i> formin	UniProtKB - Q5TJ55 (FORD_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>FORJ</i> formin	UniProtKB - Q54ER5 (FORJ_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.

<i>FOR1</i> formin	UniProtKB - Q54P19 (FOR1_DICDI)	actin cytoskeleton regulator, play an important role in the nucleation of actin and the formation of linear actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>VASP</i> ENA/VASP	UniProtKB - Q5TJ65 (VASP_DICDI)	actin cytoskeleton regulator, Ena/VASP proteins are actin-associated proteins involved in a range of processes dependent on cytoskeleton remodeling and cell polarity such as lamellipodial and filopodial dynamics in migrating cells. Plays a crucial role in filopodia formation, cell-substratum adhesion, and proper chemotaxis. Nucleates and bundles actin filaments. When complexed with forH in filopodial tips, may support formin-mediated filament elongation by bundling nascent actin filaments.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>PROB</i> profilin2	UniProtKB - P26200 (PROF2_DICDI)	actin cytoskeleton regulator, Binds to actin and affects the structure of the cytoskeleton. At high concentrations, profilin prevents the polymerization of actin, whereas it enhances it at low concentrations. By binding to PIP2, it inhibits the formation of IP3 and DG.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>PROA</i> profilin1	UniProtKB - P26199 (PROF1_DICDI)	actin cytoskeleton regulator, Binds to actin and affects the structure of the cytoskeleton. At high concentrations, profilin prevents the polymerization of actin, whereas it enhances it at low concentrations. By binding to PIP2, it inhibits the formation of IP3 and DG.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>PROC</i> profilin3	UniProtKB - Q8T8M2 (PROF3_DICDI)	actin cytoskeleton regulator, Binds to actin and affects the structure of the cytoskeleton. At high concentrations, profilin prevents the polymerization of actin, whereas it enhances it at low concentrations. By binding to PIP2, it inhibits the formation of IP3 and DG (By similarity).	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>COFA</i> cofilin1	UniProtKB - P0DJ26 (COFA_DICDI)	actin cytoskeleton regulator, Controls reversibly actin polymerization and depolymerization in a pH-sensitive manner. It has the ability to bind G- and F-actin in a 1:1 ratio of cofilin to actin. It is the major component of intranuclear and cytoplasmic actin rods (By similarity).	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>COFB</i> cofilin1	UniProtKB - P0DJ27 (COFB_DICDI)	actin cytoskeleton regulator, Controls reversibly actin polymerization and depolymerization in a pH-sensitive manner. It has the ability to bind G- and F-actin in a 1:1 ratio of cofilin to actin. It is the major component of intranuclear and cytoplasmic actin rods (By similarity)	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>CORB</i> coroninB	UniProtKB - Q55E54 (COROB_DICDI)	actin cytoskeleton regulator, Involved in phagocytosis and cell motility. Binds to actin filaments and protects them against depolymerization.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>PAKB</i> Serine/threonine-protein kinase pakB	UniProtKB - Q869N2 (PAKB_DICDI)	actin cytoskeleton regulator, Regulator of the myosin I component of the cytoskeleton: required for regulation of cytokinesis, phagocytosis and pinocytosis.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>GPBA</i> subunit beta	UniProtKB - P36408 (GBB_DICDI)	actin cytoskeleton regulator, The Gbeta null mutant displays strong reduced phagocytosis rate of bacteria, and to a lower extent latex beads, but is normal for macropinocytosis, thus providing a first evidence for signaling discriminating phagocytosis from macropinocytosis.	G Protein β Subunit-null Mutants Are Impaired in Phagocytosis and Chemotaxis Due to Inappropriate Regulation of the Actin Cytoskeleton, Peracino et al, J Cell Biol. 1998 Jun 29;141(7):1529-37., doi: 10.1083/jcb.141.7.1529
<i>CAP</i> Adenylyl cyclase-associated protein	UniProtKB - P54654 (CAP_DICDI)	actin cytoskeleton regulator, May have a regulatory bifunctional role. Binds G-actin and PIP2. Involved in microfilament reorganization near the plasma membrane in a PIP2-regulated manner.	G Protein β Subunit-null Mutants Are Impaired in Phagocytosis and Chemotaxis Due to Inappropriate Regulation of the Actin Cytoskeleton, Peracino et al, J Cell Biol. 1998 Jun 29;141(7):1529-37., doi: 10.1083/jcb.141.7.1530
<i>RASS</i>	UniProtKB - P32254 (RASS_DICDI)	Small G proteins of Ras family - molecular switches in signal td pathways and involved in regulating G protein dependent and independent processes including cell growth, cytokinesis, motility, adenylyl cyclase activation and actin polarization, null mutant - elongated actin protrusions, defective in phagocytosis and macropinocytosis.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>RAP1</i>	UniProtKB - P18613 (RAPA_DICDI)	Small G proteins of Ras family - molecular switches in signal td pathways and involved in regulating G protein dependent and independent processes including cell growth, cytokinesis, motility, adenylyl cyclase activation and actin polarization, regulateds phagocytosis, downstream effector of RasS and Rap1 is Phg2. Positively regulates phagocytosis and negatively regulates macropinocytosis. May be involved in the activation of guanylyl cyclase during the response to hyperosmotic conditions. Overexpressing cells generate alterations in cell shape and contractile responses.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>RAC1A</i>	UniProtKB - P34144 (RAC1A_DICDI)	Rac GTPases are important regulators of the actin cytoskeleton during phagocytosis, Rac1A, B and C display similar phenotypes, both in term of actin dynamics and effects on phagocytosis. Overexpression promotes the formation of filopodia and membrane ruffles.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>RAC1B</i>	UniProtKB - P34145 (RAC1B_DICDI)	Rac GTPases are important regulators of the actin cytoskeleton during phagocytosis, Rac1A, B and C display similar phenotypes, both in term of actin dynamics and effects on phagocytosis. Overexpression promotes the formation of filopodia and membrane ruffles.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>RAC1C</i>	UniProtKB - P34146 (RAC1C_DICDI)	Rac GTPases are important regulators of the actin cytoskeleton during phagocytosis, Rac1A, B and C display similar phenotypes, both in term of actin dynamics and effects on phagocytosis. Overexpression promotes the formation of filopodia and membrane ruffles.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>RACB</i>	UniProtKB - P34148 (RACB_DICDI)	Rac GTPases are important regulators of the actin cytoskeleton during phagocytosis, regulates phagocytosis	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>RACC</i>	UniProtKB - P34149 (RACC_DICDI)	Rac GTPases are important regulators of the actin cytoskeleton during phagocytosis, regulates actin dynamics through WASP activation, required for PI3K translocation to the membrane	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>RACG</i>	UniProtKB - Q9GPS0 (RACG_DICDI)	Rac GTPases are important regulators of the actin cytoskeleton during phagocytosis, targeted to the rim of the progressing phagocytic cup and disappears from the phagosome immediately after its internalization	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.
<i>RACH</i>	UniProtKB - Q9GPR7 (RACH_DICDI)	Rac GTPases are important regulators of the actin cytoskeleton during phagocytosis, severe defect in both macropinocytosis and latex beads phagocytosis was observed in cells overexpressing wild-type RacH.	Phagocytosis and host-pathogen interactions in Dictyostelium with a look at macrophages, Bozzaroet al, Int Rev Cell Mol Biol. 2008;271:253-300. doi: 10.1016/S1937-6448(08)01206-9.

6. Analyses of gene loss and gene retainment in yeast-like fungal clades.

Fisher's exact test p-values (orange: significantly more losses than expected by chance; yellow: significantly fewer losses than expected by chance)					
functional category	Saccharomycotina	Taphrinomycotina	Ustilaginomycotina	Tremellomycotina	Pucciniomycotina
actin cytoskeleton	5.30786E-09	6.91671E-06	0.153156186	0.00607406	0.002573875
adhesion	0.113324724	0.00018987	0.369256909	0.001346272	0.004584056
cell cycle	0.206123054	0.022290478	0.566002687	0.133860654	0.023525534
fungal cell wall	0.00029458	0.027425669	0.111396981	0.001292715	0.00064563
transcriptional regulators	0.001473785	0.090075022	0.28005944	0.118415438	0.143313007
microtubules	0.063333801	0.01622755	0.057295765	0.003362121	0.006695494
NADPH oxidases	0.073525897	0.274208245	0.000307352	0.225349807	0.190837848
polarity maintenance	8.20352E-05	0.017952918	0.073441071	0.037765531	0.056542222
septum formation	0.000295531	2.1592E-05	0.187082395	0.004840447	0.024151741
signaling	5.06759E-06	0.000267252	0.142175842	0.0708654	0.044422388
vesicle transport	1.02893E-13	2.60151E-07	0.015124401	3.96003E-11	3.29919E-07

Enrichment (greater than in genome-wide category means losses are enriched in that category)					
functional category	Saccharomycotina	Taphrinomycotina	Ustilaginomycotina	Tremellomycotina	Pucciniomycotina
genome-wide	0.593387178	0.551762376	0.13259997	0.539972516	0.464626335
actin cytoskeleton	0.173913043	0.234042553	0.068965517	0.314285714	0.255813953
adhesion	0.68	0.913043478	0	0.833333333	0.714285714
cell cycle	0.5	0.2	0	0.2	0
fungal cell wall	0.409638554	0.471264368	0.073170732	0.320754717	0.272727273
transcriptional regulators	0.322580645	0.46875	0.166666667	0.647058824	0.423076923
microtubules	0.647887324	0.657894737	0.032258065	0.671052632	0.580645161
NADPH oxidases	1	0.4	1	0.25	0.2
polarity maintenance	0.359375	0.442857143	0.052631579	0.435483871	0.407894737
septum formation	0.358490566	0.296875	0.108108108	0.362068966	0.359375
signaling	0.38317757	0.398305085	0.134328358	0.504672897	0.421875
vesicle transport	0.221052632	0.298969072	0.053333333	0.183908046	0.21875

Percentage of genes lost					
functional category	Saccharomycotina	Taphrinomycotina	Ustilaginomycotina	Tremellomycotina	Pucciniomycotina
genome-wide	59.33871782	55.17623762	13.25999703	53.99725164	46.46263345
cell cycle	50	20	0	20	0
vesicle transport	22.10526316	29.89690722	5.333333333	18.3908046	21.875
actin cytoskeleton	17.39130435	23.40425532	6.896551724	31.42857143	25.58139535
fungal cell wall	40.96385542	47.12643678	7.317073171	32.0754717	27.27272727
septum formation	35.8490566	29.6875	10.81081081	36.20689655	35.9375
transcriptional regulators	32.25806452	46.875	16.66666667	64.70588235	42.30769231
polarity maintenance	35.9375	44.28571429	5.263157895	43.5483871	40.78947368
signaling	38.31775701	39.83050847	13.43283582	50.46728972	42.1875
microtubules	64.78873239	65.78947368	3.225806452	67.10526316	58.06451613
adhesion	68	91.30434783	0	83.33333333	71.42857143
NADPH oxidases	100	40	100	25	20
average of lost%	45.96468486	43.47274033	15.35874872	42.93289999	35.04039783

Percentage of genes retained					
functional category	Saccharomycotina	Taphrinomycotina	Ustilaginomycotina	Tremellomycotina	Pucciniomycotina
genome-wide	40.66128218	44.82376238	86.74000297	46.00274836	53.53736655
cell cycle	50	80	100	80	100
vesicle transport	77.89473684	70.10309278	94.66666667	81.6091954	78.125
actin cytoskeleton	82.60869565	76.59574468	93.10344828	68.57142857	74.41860465
fungal cell wall	59.03614458	52.87356322	92.68292683	67.9245283	72.72727273
septum formation	64.1509434	70.3125	89.18918919	63.79310345	64.0625
transcriptional regulators	67.74193548	53.125	83.33333333	35.29411765	57.69230769
polarity maintenance	64.0625	55.71428571	94.73684211	56.4516129	59.21052632
signaling	61.68224299	60.16949153	86.56716418	49.53271028	57.8125
microtubules	35.21126761	34.21052632	96.77419355	32.89473684	41.93548387
adhesion	32	8.695652174	100	16.66666667	28.57142857
NADPH oxidases	0	60	0	75	80
average of retained%	54.03531514	56.52725967	84.64125128	57.06710001	64.95960217

Conflict of interest

As the corresponding and/or contributing author of the below mentioned peer reviewed publication I declare that the authors have no conflict of interest related to this study. I also declare that the PhD candidate Enikő Kiss worked under my supervision and had a great contribution to the overall results evidenced in this study.

Author contributions:

The candidate collected literature data on hypha morphogenesis-related genes, inferred species and gene trees, made ancestral character state reconstruction and analyzed gene family evolution. Arun N. Prasanna, Balázs Bálint and Tamás Kószó helped in inferring species trees and performing clustering. Torda Varga and Zsolt Merényi helped in statistical calculations and wrote an R script, that helped in the automatization of the visualization of gene family evolution. Botond Hegedüs helped in the analysis of gene structural changes. Sándor Kocsubé designed the hypha figure for this study. Meritxell Riquelme and Norio Takeshita helped in the interpretation of results with their great experience in hypha morphogenesis. László Nagy coordinated the whole study, helped in the analysis of phagocytosis-related and kinase gene families, and participated in the investigation of gene losses in yeast-like fungi.

Kiss, E., Hegedüs, B., Virágh, M., Varga, T., Merényi, Z., Kószó, T., Bálint, B., Prasanna, AN., Krizsán, K., Kocsubé, S., Riquelme, M., Takeshita, N., Nagy, GL, “Comparative genomics reveals the origin of fungal hyphae and multicellularity”, *Nature Communications* 2019 Sep 9;10(1):4080

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Szeged

László Nagy, Ph.D.

senior research associate

Biological Research Centre, Szeged