Abstract: Filamentous fungi typically grow as interconnected multinucleate syncytia that can be microscopic to many hectares in size. Mechanistic details and rules that govern the formation and function of these multinucleate syncytia are largely unexplored, including details on syncytial morphology and the regulatory controls of cellular and molecular processes. Recent discoveries have revealed various adaptations that enable fungal syncytia to accomplish coordinated behaviors, including cell growth, nuclear division, secretion, communication, and adaptation of the hyphal network for mixing nuclear and cytoplasmic organelles. In this review, we highlight recent studies using advanced technologies to define rules that govern organizing principles of hyphal and colony differentiation, including various aspects of nuclear and mitochondrial cooperation versus competition. We place these findings into context with previous foundational literature and present still unanswered questions on mechanistic aspects, function, and morphological diversity of fungal syncytia across the fungal kingdom.

Keywords: syncytia; filamentous fungi; heterokaryon; nucleus; morphology

1. Syncytia

Syncytia can be defined as multinucleated cells within a common cytoplasmic environment whose main purpose is to function as a single coordinated unit. There are many cases of syncytia in nature. In animals, for example, the placenta is a multinucleate organ that has an important role in protecting the fetus and transporting nutrients [1]. In muscle cells, multinucleated fibers are formed via the fusion of myoblasts [2], and osteoclasts are multinuclear bone-resorbing cells that are formed via cell fusion [3] (Figure 1a). In the slime mold, Physarum polycephalum, individual amoebae fuse to form a large mass of protoplasm containing multiple nuclei [4] that undergo synchronous mitoses [5] (Figure 1b). In plants, syncytia also occur, for example, the endosperm–placental syncytia [6] (Figure 1c). In fungi, diversity of morphological characteristics that have evolved over time, and the syncytial lifestyle may have arisen in hyphal and non-hyphal organisms. For example, chytrids species belong to an ancient phylum of fungi (Chytridiomycota) that diverged at least 750 million years ago from other fungal lineages [7]. Chytrids do not form long multinucleate hyphae that search for nutrients. Instead, they have anucleate ‘rhizoids’ that are derived from a unicellular, multinucleate structure, or more complex rhizomycelia, which share morphological features with filamentous fungal syncytia and multinucleate hyphae.
Figure 1. Examples of syncytia in nature. (a) Myoblasts are fused to form multinucleated muscle fibers. (b) Slime mold *Physarum polycephalum*, multinucleate protoplasm formed by the fusion of individual amoebae. (c) Endosperm–placental syncytia in developmental stages of *Urticularia* seeds, the formation of syncytia occurs in the placenta. The syncytia harbor two populations of nuclei, a nucleus from the nutritive tissue, and a giant endosperm nucleus (modified from [8]).

The hallmark growth habit of filamentous fungi is as multinucleate syncytia (Figure 2a,b). The interconnectedness of fungal syncytia occurs via fusion of germinated asexual spores (germlings) via so-called conidial anastomosis tubes (CATs) [9] or between hyphae within a single colony or between colonies (anastomosis) [10] (Figure 2a–d). Fusion results in the formation of an interconnected network (mycelium) [11,12], which is the foundation for colony establishment and growth. In *Neurospora crassa*, anastomosis is associated with cell-to-cell communication processes that regulate chemotropic growth of germlings and hyphae before cell contact [10]. Germlings/hyphae send and receive signals that guide chemotropic interactions, culminating in cell–cell adhesion, cell wall dissolution, membrane merger, and cytoplasmic mixing [13–16]. Hyphal anastomosis primarily occurs in the interior of a colony either through hyphal branches or by contact between adjacent hyphae, resulting in the formation of a fusion bridge [10,11]. Hyphal fusion can also occur between colonies with the same or non-identical genotypes [17,18].

The formation of an interconnected syncytium can be beneficial for colony development, facilitating the exchange of genetic material, nutrient transport, improving colony establishment, and increasing colony size [17,19–21]. Upon hyphal fusion, cytoplasmic flow can show dramatic changes in directionality [10,22]. Germlings or young colonies can share genetic resources or nutrients through cell fusion, but the process can be restricted as colonies age and undergo hyphal differentiation [17]. The advantages of interconnected syncytial hyphae lie in their ability to transport nutrients through a continuous cytoplasm, allowing the efficient use of the nutrients once a local source has been exhausted [23].

Within a syncytium, heterokaryons can arise due to a spontaneous mutation within a single colony (Figure 3a). In a laboratory strain of *N. crassa*, around 2–3% of the nuclear population harbored mutations that modified the mycelium phenotype, showing the difficulties of maintaining a mycelium with a uniform genetic background [24]. Heterokaryons can also form via germling or hyphal fusion between genetically different cells/colonies, resulting in the coexistence of genetically
different nuclei in a common cytoplasm (Figure 2e,f). Heterokaryon formation is an essential element of many fungal life cycles and may be useful to complement mutations. There are also beneficial aspects of the parasexual cycle, such as functional diploidy and mitotic recombination [25] (Figure 3d). However, heterokaryon formation can transmit genetic infections that negatively impact fitness, such as the transmission of mycoviruses [26,27], deleterious mitochondrial DNA, and senescence plasmids [28,29], transposons [30], or parasitic nuclei [31]. In this case, heterokaryon formation and viability are regulated by ‘self/non-self’ or ‘allorecognition mechanisms’ via genetic differences at \textit{het} (heterokaryon) or \textit{vic} (vegetative incompatibility) loci [26,32–35]. In \textit{N. crassa}, allorecognition and restriction of heterokaryon formation has been dissected and can be summarized in three key points: (i) cells that differ in allorecognition specificity at the \textit{doc} (determinate of communication) loci restrict chemotropic interactions to cells with identical specificity at the \textit{doc} loci [36], (ii) cells that differ in allorecognition specificity at the \textit{cwr} (cell wall remodeling) loci, undergo chemotropic interactions, but cell wall dissolution of adhered cells is blocked [37], and (iii) cells that perform chemotropic interactions and cell fusion undergo a programmed cell death reaction following fusion that restricts cytoplasmic mixing of the two cells/hyphae if they differ in allorecognition specificity at \textit{het} loci [32,38–41] (Figure 2e–h). Within an \textit{N. crassa} population, a very large number of incompatible genotypes are possible upon segregation of \textit{doc}, \textit{cwr}, and \textit{het} loci, making heterokaryon formation between cells of different genetic backgrounds highly unlikely [41,42].

**Figure 2.** Formation of interconnected fungal syncytia. (a) The fusion of compatible strains of \textit{Neurospora crassa}, whose nuclei are labeled with either histone H1-GFP or H1-dsRED (green and magenta, respectively). (b) Genetically identical germs grow to each other (dashed arrows), fuse, and give rise to interconnected multinucleate syncytia. (c) Heterogeneity of the mycelial network and syncytia formation. Magenta dots are nuclei marked with histone H1-DsRed. The box shows a close-up of a hypha, showing the marked nuclei. (d) Hyphal fusion within a colony contributes to an interconnected syncytium. (e) In hyphae, heterokaryon formation can occur when there are no differences at \textit{het} (heterokaryon) or \textit{vic} (vegetative incompatibility) loci. In contrast, genetic differences at these loci result in heterokaryon incompatibility, which triggers compartmentalization of the fusion compartment due to occlusion of the septum, vacuolization of the hyphae, and eventual cell death. (f) In germlings, heterokaryon formation can occur when there are no differences at \textit{red-1} (regulator of cell death-1) and \textit{plp-1/sec-9} (patatin-like phospholipase-1) loci. In contrast, differences at these loci result in heterokaryon incompatibility, rapidly triggering a cell death reaction that is a similar process in hyphae [38,39]. (g) Micrographs show the fusion of compatible germlings. One of the germlings is marked with cytoplasmic Green Fluorescent Protein (GFP) (green) and has undergone cell fusion with a compatible germling stained with FM4-64 fluorescent dye (red). Fusion
is evident by the fact that GFP fluorescence can be observed in both germlings due to cytoplasmic mixing, and cell death does not occur, as indicated with the absence of SYTOX Blue fluorescence (death cell stain). (h) Cell fusion between germlings with genetic differences at the plp/sec-9 loci results in rapid cellular vacuolization and death, as demonstrated with the staining of SYTOX Blue fluorescence. White arrows indicate fusion events. Micrographs also show two germlings that have not undergone cell fusion and are healthy (green; GFP and red arrows; FM4-64). Micrographs (g,h) courtesy of Dr. Jens Heller (UCB Glass Laboratory).

2. Differentiation within Fungal Syncytia

2.1. Hyphal Architecture

A fungal syncytium is in contact with the surrounding environment and encounters a variety of external stimuli that require precise and, oftentimes, localized responses. Physiological specialization of hyphae within a colony can partition tasks within the syncytium. Observations of heterogeneous hyphal architecture, such as a morphological transition to thicker diameter ‘trunk hyphae’, differences in hyphal compartment sizes, extension rates, and branching associated with the anatomy of fungal syncytia, have been described [43–47] (Figure 2c). Heterogeneity of syncytial architecture is not limited by hyphal diameter, but also by differences in septation [48] and mobility of organelles, such as nuclei and mitochondria [10,22]. Studies in Aspergillus niger showed heterogeneous unidirectional transport of sugar analogs from the colony center to the periphery of the colony, as well as the autonomy of apical compartments (vs. subapical and basal compartments), based on the ability to rapidly plug lysed cells via Woronin bodies [49–51]. Recent experiments in the basidiomycete species, Coprinopsis cinerea, revealed the presence of specialized trunk hyphae that transported defense response compounds and nutrients bidirectionally across the fungal colony in response to localized fungivory by nematodes, while smaller hyphae exhibited a unidirectional flow [52]. Hyphae can also serve as building blocks for more complex or differentiated hyphal arrangements in basidiomycetes and some ascomycete species, called ‘rhizomorphs’ or ‘mycelial cords’. Rhizomorph is a term used for growing hyphal tips with apical dominance, and mycelial cords are aggregations of up to thousands of younger (and oftentimes melanized) parallel bundled hyphae that are fused by anastomosis to an older, leading hypha [53–55]. These rhizomorphs and mycelial cords are highly differentiated and share many structural components that resemble plant roots, such as a small region behind the growth front that undergoes rapid cell division and layers of cells that guard the growing cords against damage by soil particles [56]. These differentiated structures can grow many times faster than normal hyphae and can form linkages between nutrient-poor and nutrient-rich substrates, as well as transporting water and nutrients across large distances.

2.2. Colony Aging

The Kingdom Fungi contains some of the longest-living organisms on the planet. The ‘humongous fungus’, or Armillaria gallica, is an example of a fungal syncytium that has lived for over 2500 years, covers 37 hectares, and weighs more than 4 × 10^6 kg [57]. Even through time and distance, the genetic makeup of this single A. gallica colony has remained surprisingly stable. The diversity of habitats and time scales in which fungi have colonized the planet suggests that fungal syncytia undergo a variety of morphological changes as the mycelium ages. It has been hypothesized that some iteration of ‘paramorphogenetic compounds’ in the center of the colony accumulate when the mycelium reaches a certain age, and this threshold could be a primary driver of hyphal differentiation spatially and temporally (as opposed to being primarily nutrient-based) [47]. Changes in colony architecture are also associated with young colonies. In N. crassa, 22 h following asexual spore germination, branch angles change from 90 degrees to 66 degrees, and by 40 and 44 h, hyphal diameters and extension rates plateau, respectively [47]. In Ashbya gossypii, chromosome number and ploidy varies as the colony ages [58]. Cytoplasmic streaming can be prevented in hyphal networks by plugging of septal pores by Woronin bodies or other septal pore proteins, thus preventing cytoplasmic streaming throughout the hyphal network [59,60]. Heterogeneity in the older parts of
the colony has been postulated to prevent the systemic spread of pathogens, therefore, maintaining the health of the colony overall [50]. Another process associated with aging colonies is autophagy. This process is integral to recycle nutrients and organelles from older parts of a syncytium to facilitate new growth [61].

One aspect of colony aging is the response to stress in the environment. Although relatively little is known about how syncytium formation directly drives stress response on a cellular or organismal level, there have been several studies suggesting there are benefits of syncytia in dealing with stress. Increased tolerance to variation in ploidy level has been observed in *Ashbya gossypii* cells [62]; homogenization of various nucleotypes and macromolecules also occurs through cytoplasmic flow [22,63]. In addition, compartmentalization of damage and regeneration of growing tips has also been shown to be an important aspect of syncytial morphology in *Aspergillus niger* [51].

2.3. Heterogeneity between Hyphal Compartments

The advent of sequencing capabilities and bioinformatics pipelines has made techniques for analyzing subtle differences in transcriptional or translational programs between hyphae possible [64]. The coupling of techniques, such as laser capture microdissection and RNA-seq [65], has been used to isolate specific fungal tissues to assess differences in gene expression. In *Aspergillus niger*, laser capture microdissection and single-cell profiling of hyphal tips in close proximity revealed expression differences between hyphae [66]. Similar experiments between neighboring hyphae revealed heterogeneous secretion of enzymes for carbohydrate acquisition, such as glucoamylase [67]. In *N. crassa*, expression profiling of a sectioned colony from peripheral to internal portions showed spatially distinct mRNA expression patterns. At the periphery of the colony, genes related to polarized growth, biosynthesis of the cell membrane, and cellular signaling were more highly expressed, while for the middle section, genes implicated in energy production showed an increase in expression level [68]; these results were comparable to those found for *A. niger* [69]. These results highlight the fact that in fungal syncytia, different hyphal types occur, and differential gene regulation and important cellular functions can be spatially and temporally regulated across a colony. However, the molecular mechanism and rules whereby this differentiation and differential gene regulation occurs within a syncytium remain obscure.

2.4. Nuclear Competition and Cooperation

Genetically distinct nuclei existing in the same cellular space are found in many filamentous fungal syncytia [70,71], although mechanisms governing nuclear coordination and competition in heterokaryons are largely unknown. A long-standing hypothesis is that by sharing a common cytoplasm in syncytia, nuclei would act in concert for the production/utilization of ‘common goods’. Furthermore, heterokaryons containing genetically distinct haploid nuclei would be functionally equivalent to diploid organisms (Figure 3b); expression profiling in the mushroom species, *Agaricus bisporus*, showed nuclear-specific expression patterns that were associated with the nuclei harboring different mating types [72]. In filamentous ascomycete species, genetic diversity within a colony can be generated via parasexual genetics or the transmission of genetic material between nuclei in the absence of mitosis or meiosis (Figure 3d). The parasexual cycle has been observed in many haploid filamentous fungi and is routinely used for genetic linkage mapping [25,73,74]. Despite incompatibility reactions between strains that affect the ability to form viable heterokaryons, under certain circumstances, these internuclear genetic interactions provide additional chances for mutually beneficial DNA elements to be transferred and utilized by genetically distinct nuclei for the benefit of the syncytium. ‘Accessory’, or ‘dispensable chromosomes’, often contain virulence genes, and through chromosomal rearrangements, spontaneous pathogenic strains can arise from otherwise non-pathogenic isolates [75]. In studies of *Fusarium* spp. and *Nectria haematococa*, heterokaryotic syncytia were shown to exchange and retain chromosomes/genes between nuclei associated with virulence, while in some cases eliminating those not involved in pathogenicity [76–79]. In *Saccharomyces cerevisiae* cells, which do not usually form heterokaryons, a small portion of the mating population (~1%) may form a transient type of heterokaryon, termed a ‘cytoductant’. Cytoductants
undergo conventional DNA and mitochondrial genetic recombination as seen in normal zygotes, but also show chromosome transfer, which suggests that this could be a fundamental property of fungi that plays a role in providing genetic variation in a population [80].

One caveat with these concepts is that nuclei within syncytia also potentially undergo competition, whereby a nucleus with a clear fitness advantage could dominate over less advantaged nuclei throughout a heterokaryotic colony [12] (Figure 3c). A major technical issue for filamentous fungal researchers tackling these questions is the identification and visualization of genetically distinct nuclei within syncytia. Fluorescent dyes and fluorophore-tagged nuclear proteins are often readily exchanged between nuclei [22,81,82]. Previous studies with filamentous ascomycete species showed that environmental pressures can drive unbalanced nuclear ratios [83,84]. Systematic studies using microsatellite markers for the analysis of unbalanced nuclear ratios in basidiomycete species also revealed that the representation of a genotype can be augmented by environmental pressures [85,86]. In many heterokaryon combinations, one nucleus dominated in terms of representation, suggesting inherent genetic fitness, in addition to environmental pressure, can drive heterogeneity of nuclear ratios within a syncytium. Mutations in a subset of nuclei within a homokaryon can also create heterokaryons, and those mutations can be beneficial or unfavorable to the fitness of the syncytium [85,87]. In some cases, syncytia may contain ‘senescent nuclei’, which despite imparting clear morphological defects and being detrimental to the colony as a whole, they are able to overproliferate, either due to faster replication or by other means [88,89]. These data suggest additional levels of regulation could contribute to how genotypic autonomy is regulated, such as the time a nucleus (or mitochondria) spends in a transcriptional versus replicative state.

Access by nuclei to asexual or sexual spores during conidiation or before meiosis could also promote nuclear competition [90]. Recent work with natural mating-type heterokaryotic species, Neurospora tetrasperma, showed a clear bias for selection by mating-type (mat A) and associated genes during vegetative growth and asexual development, although ratios of mat A and mat a nuclei equalized during sexual development [91]. Heterokaryons of the basidiomycete species Heterobasidion parviporum consisting of sibling-composed nuclei tended to produce nuclear distributions and germination rates of asexual spores that were very similar to homokaryons. However, heterokaryons composed of non-sibling related nuclei tended to form uninucleate conidia that germinated faster than those that were bi- and trinucleate from non-sibling heterokaryons.

2.5. Nuclear Autonomy

Nuclei maintain autonomy during cellular events that take place in a common cytoplasm in filamentous fungi, oftentimes at relatively short internuclear distances, such as asynchronous mitosis [92,93] and parasynchronous mitosis [94,95] (Figure 3e). A number of hypotheses for asynchronous cell cycle progression have been proposed in A. gossypii: (i) subsets of nuclei could emit cell cycle blocking molecules, such as CDK inhibitors, locally restricting cell cycle progression of neighboring nuclei; (ii) seemingly random distribution of factors which positively promote cell cycle progression, in addition to varying requirements for each nucleus to enter the cell cycle may lead to asynchronicity; (iii) positioning of nuclei in relation to cortical markers, which may exhibit very specific localization, could determine the fate of nuclei to enter the cell cycle; (iv) nuclei and associated transcripts/proteins may be separated in the cytoplasm, for example, organelles or membranes could partition translated proteins to a specific part of a nucleus or subset of nuclei; (v) cytoskeletal elements, nuclear pore proteins, or transcription factors may be asymmetrically distributed between ‘mother’ and ‘daughter’ nuclei upon mitotic exit, thereby mis-synchronizing future cell cycle timing [93].

Understanding is also still lacking about the positioning and fate of mRNA transcripts and proteins within a syncytial cell, relative to the nucleus of origin. Recent studies involving microtubule-associated nuclear repulsion [96], as well as cytoplasmic streaming and ‘eddy currents’ [59] suggest that ‘nuclear neighborhoods’ occur in multinucleate hyphae, and microenvironments in the cytoplasm regulate where nuclei, organelles, and cytoskeletal elements aggregate, affect transcriptional patterns and access local pools of ‘common goods’. These ‘neighborhoods’ could be
partially due to a shift in the physical state of the cytoplasm to a gel-like conformation (Figure 3g), mediated by a phase separation via unstructured regions of polypeptides, as has been shown in vitro [97–99]. In A. gossypii, mechanistic details connecting the cell cycle and nuclear coordination were assisted by adapting single-molecule fluorescence in-situ hybridization (smFISH) protocols to visualize mRNA transcripts of mitotic cyclins in fixed cells. PolyQ-driven assemblies of protein-RNA were found to affect the spatial distribution of transcripts for the cell cycle regulator CLN3 by facilitating a shift in physical state, followed by aggregation with complexes of similar physical properties, both in-vivo and in-vitro [100–103]. These observations point to how the partitioning of the nucleoplasm and cytoplasm can facilitate asynchronous and parasynchronous cellular events (Figure 3e).

The ratio of nuclei to cytoplasm also seems to vary across the fungal kingdom. In A. gossypii, it has been shown that the ratio of nuclei per unit volume of cytoplasm (#N/C) remains constant even when nuclei are clustered, as is the case with the mutant defective in the function of dynactin (jnm1Δ) [102]. Dynein mutants in several fungal species lead to clusters of nuclei, indicating a role for dynein in nuclear distribution and migration in hyphae [104–106]. Nuclear repulsion facilitates sufficient spacing between nuclei to allow for a stable cytoplasmic–nuclear ratio throughout growth (Figure 3f), despite exhibiting asynchronous mitoses [93,96]. Upon removal of nocodazole, which blocks nuclear division, nuclei underwent rapid divisions to reestablish wildtype-level internuclear spacing and cytoplasmic–nuclear ratios [96]. In contrast, although N. crassa also exhibit asynchronous mitoses, fixed internuclear distances are not maintained throughout growth [22]. Slime molds like P. polycephalum, which share many similar properties with fungal syncytia, undergo synchronous mitosis, where the entire nuclear and cytoplasmic contents double together [107]. Aspergillus nidulans undergoes a parasynchronous mitotic wave, where the more apical compartments undergo rapid extension accompanied by faster nuclear division, while more basal compartments remain mitotically inactive or less active [108]. In this case, branching was correlated with nuclear division and an increase in cytoplasmic volume. In Schizosaccharomyces pombe, the cell maintains a nuclear size proportional to cell size (N/C ratio) [109]. Mutants with defects in nucleocytoplasmic mRNA transport and lipid synthesis were altered in their N/C ratio, indicating that cells must regulate nucleocytoplasmic transport and nuclear membrane growth to maintain appropriate N/C ratios within the cell [110]. Much remains to understand the regulatory mechanisms associated with nuclear autonomy and its integration with cellular processes, in particular, how unicellular and syncytial fungi ‘sense’ and respond to fluctuations in cytoplasmic volume and nuclear content under different environments and growth habits.

**Figure 3.** Nuclear patterns in multinucleate syncytia. (a) Spontaneous mutation in nuclei can give that nucleus an advantage or disadvantage over the rest of the nuclei in the syncytial population. (b)
Nuclear complementation can occur if a nucleus lacks a gene (x- or y-) encoding a function necessary for survival. x- or y-function can be complemented by the presence of a second nucleus, which is functional for that gene (x+ or y+). Complementation between nuclei in a syncytium can occur with spontaneous mutations (a) or via complementation upon fusion with another individual that can produce the missing component. (e) In multinucleate syncytia, variations in the nuclear ratios can occur, where one nucleus can dominate. (d) Generation of nuclear heterogeneity through the parasexual cycle. Haploid nuclei in a heterokaryon, formed either by spontaneous mutation or via fusion with a different strain, undergo karyogamy to form a heterozygous diploid nucleus. Repeated rounds of mitotic recombination and mitotic nondisjunction result in loss of chromosomes, producing haploid nuclei with unique genotypes. (e) Different patterns of nuclei division in syncytia. Synchrony: all the nuclei divide at the same time. Parasynchrony, the mitosis is initiated in one nucleus, and then linearly, the adjacent nucleus starts to divide after the first one. Asynchrony, the nuclei divide independently of each other (modified from [93]). (f) The nuclei show repulsion to delimit their cytoplasmic territory [96], and within a hypha, a regular number of nuclei per unit volume of cytoplasm (×N/C) is observed [102]. (g) Nuclear neighborhoods can be organized around nuclei that affect cell cycle regulation and, potentially, other regulatory processes (modified from [111]).

2.6. Mitochondrial Autonomy

Although the nuclear genome is thought of as being the primary source of genomic information in the cell, mitochondria also contain a distinct genome. In filamentous fungi, the transmission of mitochondria solely by one parent (in ascomycetes, this is primarily the ‘maternal’ parent, regardless of mating-type) can occur during sexual reproduction [112–114]. In N. tetrasperma, mitochondria show a ‘maternal’-only inheritance via fertilization with specialized mating hyphae (trichogynes). However, in cases where mating was initiated by hyphal fusion, one mitochondrial DNA fully replaced the mitochondrial DNA of the hyphal fusion partner [113]. In heterothallic basidiomycete species, there is an ordered exchange of nuclei, but not cytoplasm between vegetative cells of monokaryons, creating a dikaryon with a homogenous distribution of two genetically distinct nuclei per cellular compartment, whereas a homogenous or heterogeneous mix of mitochondrial genomes can occur. This creates a ‘patchwork’ of mitochondrial genotypes throughout the mycelial dikaryon [115].

3. Conclusion and Outlook

Despite decades of research on fungal syncytia, we have only just begun to elucidate many mechanistic details of how function and proliferate. There appears to be far more nuclear and mitochondrial autonomy and natural partitioning of cellular compartments than previously hypothesized within fungal syncytia. Despite the interconnectedness of filamentous fungal networks, researchers have unveiled a picture of a more heterogeneous landscape of gene expression, metabolic function, and protein production, both spatially and temporally, within growing syncytia. Future work in this area of research should address some of the unanswered questions, such as (i) What are the basic rules that govern fungal syncytia formation and viability? (ii) How and to what extent do genetically distinct nuclei and mitochondria coordinate and/or compete with one another within a fungal syncytium? (iii) what internal/external stimuli govern differentiation into particular cell types as a colony ages? (iv) Evolutionarily-speaking, how and why did the transition from unicellularity to a syncytial lifestyle develop across the Kingdom Fungi? Considering filamentous fungi are currently used as the ‘workhorses’ of modern industrial production of compounds, and there has been a multitude of fungal-derived materials and bio-products manufactured in recent years, understanding these questions has implications for engineering syncytial fungi for optimal protein production in industrial settings. Future studies in this field will also improve our understanding of how large hyphal networks (>15 hectares) affect carbon cycling and nutrient translocation in the environment.
**Author Contributions:** A.P.M. writing, review and editing; A.M.R.-R. writing, review, and figures design; N.L.G. writing, review, and editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** A.P.M. is funded by a RoL-FELS-RAISE grant from the National Science Foundation (1840273), A.M.R.-R. is funded by a National Science Foundation grant (1818283). N.L.G. is partially funded by Fred E. Dickinson Chair of Wood Science and Technology.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Zeldovich, V.B.; Clausen, C.H.; Bradford, E.; Fletcher, D.A.; Maltepe, E.; Robbins, J.R.; Bakardjiev, A.I. Placental syncytium forms a biophysical barrier against pathogen invasion. *PLoS Pathog*. 2013, 9, e1003821.
2. Abmayr, S.M.; Zhuang, S.; Geisbrecht, E.R. Myoblast fusion in Drosophila. *Methods Mol. Biol.* 2008, 475, 75–97.
3. Scheven, B.A.; Haas, E.W.K-D.; Wassenaar, A.M.; Nijweide, P.J. Differentiation kinetics of osteoclasts in the periosteum of embryonic bones in vivo and in vitro. *Anat. Rec.* 1986, 214, 418–423.
4. Daniel, J.W.; Jarflors, I. Plasmodial ultrastructure of the myxomycete *Physarum polycephalum*. *Tissue Cell* 1972, 4, 15–36.
5. Guttes, E.; Guttes, S.; Rusch, H.P. Morphological observations on growth and differentiation of *Physarum polycephalum* grown in pure culture. *Dev. Biol.* 1961, 3, 588–614.
6. Plachno, B.J.; Świątek, P. Syncytia in plants: Cell fusion in endosperm-placental syncytium formation in Utricularia (Lentibulariaceae). *Protoplasma* 2011, 248, 425–435.
7. Laundon, D.; Chrismas, N.; Wheeler, G.; Cunliffe, M. Chytrid rhizoid morphogenesis resembles hyphal development in multicellular fungi and is adaptive to resource availability. *Proc. Biol. Sci.* 2020, 287, 20200433.
8. Plachno, B.J.; Świątek, P.; Sas-Nowosielska, H.; Kozieradzka-Kiszkonu, M. Organisation of the endosperm and endosperm-placenta syncytia in bladerworts (Utricularia, Lentibulariaceae) with emphasis on the microtubule arrangement. *Protoplasma* 2013, 250, 863–873.
9. Roca, G.M.; Read, N.D.; Wheals, A.E. Conidial anastomosis tubes in filamentous fungi. *FEMS Microbiol. Lett.* 2005, 249, 191–198.
10. Hickey, P.C.; Jacobson, D.; Read, N.D.; Glass, N.L. Live-cell imaging of vegetative hyphal fusion in *Neurospora crassa*. *Fungal Genet. Biol.* 2002, 37, 109–119.
11. Glass, N.L.; Fleißner, A. Re-wiring the network: Understanding the mechanism and function of anastomosis in filamentous ascomycete fungi. In *The Mycota*; Kues, U., Ed.; Springer: Berlin/Heidelberg, Germany, 2006; pp. 123–139.
12. Rayner, A.D.M. *Interconnectedness and Individualism in Fungal Mycelia*; Cambridge University Press: Cambridge, UK, 1996; pp. 193–232.
13. Fischer, M.S.; Glass, N.L. Communicate and fuse: How filamentous fungi establish and maintain an interconnected mycelial network. *Front. Microbiol.* 2019, 10, 619.
14. Herzog, S.; Schumann, M.R.; Fleissner, A. Cell fusion in *Neurospora crassa*. *Curr. Opin. Microbiol.* 2015, 28, 53–59.
15. Goryachev, A.B.; Lichius, A.; Wright, G.D.; Read, N.D. Excitable behavior can explain the “ping-pong” mode of communication between cells using the same chemoattractant. *Bioessays* 2012, 34, 259–266.
16. Fleissner, A.; Leeder, A.C.; Roca, M.G.; Read, N.D.; Glass, N.L. Oscillatory recruitment of signaling proteins to cell tips promotes coordinated behavior during cell fusion. *Proc. Natl. Acad. Sci. USA* 2009, 106, 19387–19392.
17. Simonin, A.; Palma-Guerrero, J.; Fricker, M.; Glass, N.L. Physiological significance of network organization in fungi. *Eukaryot. Cell* 2012, 11, 1345–1352.
18. Glass, N.L.; Dementhon, K. Non-self recognition and programmed cell death in filamentous fungi. *Curr. Opin. Microbiol.* 2006, 9, 553–558.
19. Bastiaans, E.; Debets, A.J.; Aanen, D.K. Experimental demonstration of the benefits of somatic fusion and the consequences for allorecognition. *Evolution* 2015, 69, 1091–1099.
20. Leeder, A.C.; Jonkers, W.; Li, J.; Glass, N.L. Germination and early colony establishment in *Neurospora crassa* requires a MAP kinase regulatory network. *Genetics* 2013, 195, 883–898.
21. Richard, F.; Glass, N.L.; Pringle, A. Cooperation among germinating spores facilitates the growth of the fungus, *Neurospora crassa*. *Biol. Lett.* 2012, 8, 419–422.

22. Roper, M.; Simonin, A.; Hickey, P.C.; Leeder, A.; Glass, N.L. Nuclear dynamics in a fungal chimera. *Proc. Natl. Acad. Sci. USA* 2013, 110, 12875–12880.

23. Heaton, L.L.M.; Jones, N.S.; Fricker, M.D. A mechanistic explanation of the transition to simple multicellularity in fungi. *Nat. Commun.* 2020, 11, 2594.

24. Maheshwari, R. Nuclear behavior in fungal hyphae. *FEMS Microbiol. Lett.* 2005, 249, 7–14.

25. Pontecorvo, G. The parasexual cycle in fungi. *Annu. Rev. Microbiol.* 1956, 10, 393–400.

26. Leslie, J.F. Fungal vegetative compatibility. *Annu. Rev. Phytopathol.* 1993, 31, 127–150.

27. Bastiaans, E.; Aanen, D.K.; Debets, A.J.; Hoekstra, R.F.; Lestradé, B.; Maas, M.F. Regular bottlenecks and restrictions to somatic fusion prevent the accumulation of mitochondrial defects in *Neurospora*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2014, 369, 20130448.

28. Debets, F.; Yang, X.; Griffiths, A.J. Vegetative incompatibility in *Neurospora*: Its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations. *Curr. Genet.* 1994, 26, 113–119.

29. Caten, C.E. Vegetative incompatibility and cytoplasmic infection in fungi. *J. Gen. Microbiol.* 1972, 72, 221–229.

30. Kinsey, J.A. Tad, a LINE-like transposable element of Neurospora, can transpose between nuclei in heterokaryons. *Genetics* 1990, 126, 317–323.

31. Debets, A.J.M.; Griffiths, A.J.F. Polymorphism of *het*-genes prevents resource plundering in *Neurospora crassa*. *Mycol. Res.* 1998, 102, 1343–1349.

32. Saupe, S.J. Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. *Microbiol. Mol. Biol. Rev.* 2000, 64, 489–502.

33. Goncalves, A.P.; Heller, J.; Daskalov, A.; Videira, A.; Glass, N.L. Regulated forms of cell death in fungi. *Front. Microbiol.* 2017, 8, 1837.

34. Glass, N.L.; Jacobson, D.J.; Shiu, P.K. The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. *Annu. Rev. Genet.* 2000, 34, 165–186.

35. Daskalov, A.; Mitchell, P.S.; Sandstrom, A.; Vance, R.E.; Glass, N.L. Molecular characterization of a fungal gasdermin-like protein. *Proc. Natl. Acad. Sci. USA* 2020, 117, 18600–18607.

36. Heller, J.; Zhao, J.; Rosenfield, G.; Kowbel, D.J.; Gladieux, P.; Glass, N.L. Characterization of greenbeard genes involved in long-distance kind discrimination in a microbial eukaryote. *PLoS Biol.* 2016, 14, e1002431.

37. Goncalves, A.P.; Heller, J.; Span, E.A.; Rosenfield, G.; Do, H.P.; Palma-Guerrero, J.; Requena, N.; Marletta, M.A.; Glass, N.L. Allorecognition upon fungal cell-cell contact determines social cooperation and impacts the acquisition of multicellularity. *Curr. Biol.* 2019, 29, 3006–3017.e3003.

38. Daskalov, A.; Gladieux, P.; Heller, J.; Glass, N.L. Programmed cell death in *Neurospora crassa* is controlled by the allorecognition determinant *rac-1*. *Genetics* 2019, 213, 1387–1400.

39. Heller, J.; Clave, C.; Gladieux, P.; Saupe, S.J.; Glass, N.L. NLR surveillance of essential SEC-9 SNARE proteins induces programmed cell death upon allorecognition in filamentous fungi. *Proc. Natl. Acad. Sci. USA* 2018, 115, E2292–E2301.

40. Daskalov, A.; Heller, J.; Herzog, S.; Fleissner, A.; Glass, N.L. Molecular mechanisms regulating cell fusion and heterokaryon formation in filamentous fungi. *Microbiol. Spectr.* 2017, 5. doi:10.1128/microbiolspec.FUNK-0015-2016.

41. Goncalves, A.P.; Glass, N.L. Fungal social barriers: To fuse, or not to fuse, that is the question. *Commun. Integr. Biol.* 2020, 13, 39–42.

42. Muirhead, C.A.; Glass, N.L.; Slatkin, M. Multilocus self-recognition systems in fungi as a cause of trans-species polymorphism. *Genetics* 2002, 161, 633–641.

43. Steele, G.C.; Trinci, A.P. Morphology and growth kinetics of hyphae of differentiated and undifferentiated mycelia of *Neurospora crassa*. *J. Gen. Microbiol.* 1975, 91, 362–368.

44. Bistis, G.N.; Perkins, D.D.; Read, N.D. Different cell types in *Neurospora crassa*. *Fungal Genet. Newslett.* 2003, 50, 17–19.

45. Lew, R.R. Mass flow and pressure-driven hyphal extension in *Neurospora crassa*. *Microbiology* 2005, 151, 2685–2692.

46. Harris, S.D. Hyphal branching in filamentous fungi. *Dev. Biol.* 2019, 451, 35–39.

47. McLean, K.M.; Prosser, J.I. Development of vegetative mycelium during colony growth of *Neurospora crassa*. *Trans. Br. Mycol. Soc.* 1987, 88, 489–495.
Hayakawa, Y.; Ishikawa, E.; Shoji, J.Y.; Nakano, H.; Kitamoto, K. Septum-directed secretion in the filamentous fungus *Aspergillus oryzae*. *Mol. Microbiol.* **2011**, *81*, 40–55.

Bleichrodt, R.J.; van Veluw, G.J.; Recter, B.; Maruyama, J.; Kitamoto, K.; Wosten, H.A. Hyphal heterogeneity in *Aspergillus oryzae* is the result of dynamic closure of septa by Woronin bodies. *Mol. Microbiol.* **2012**, *86*, 1334–1344.

Bleichrodt, R.J.; Vinck, A.; Read, N.D.; Wosten, H.A. Selective transport between heterogeneous hyphal compartments via the plasma membrane lining septal walls of *Aspergillus niger*. *Fungal Genet. Biol.* **2015**, *82*, 193–200.

Tegelaar, M.; Wosten, H.A.B. Functional distinction of hyphal compartments. *Sci. Rep.* **2017**, *7*, 6039.

Schmieder, S.S.; Stanley, C.E.; Rzepiela, A.; van Swaay, D.; Sabotic, J.; Norrelykke, S.F.; deMello, A.J.; Aebi, M.; Kunzler, M. Bidirectional propagation of signals and nutrients in fungal networks via specialized hyphae. *Curr. Biol.* **2019**, *29*, 217–228.e214.

Moore, D. Tissue Formation. In *The Growing Fungus*; Gow, N.A.R., Gadd, G.M., Eds.; Chapman & Hall: London, UK, 1994; pp. 423–465.

Watkinson, S.C.; Boddy, L.; Money, N.P. *The Fungi*, 3rd ed.; Academic Press: USA, 2016; p. 466.

Yaletto, L. The structure of mycelial cords and rhizomorphs in fungi: A mini review. *Mycosphere* **2018**, *9*, 984–998.

Watkinson, S. Growth of rhizomorphs, mycelial strands, coremia and sclerotia. In *Fungal Walls and Hyphal Growth*; Burnett, J.H., Trinci, A.P.J., Eds.; Cambridge University Press, Cambridge, 1979; pp. 93–113.

Anderson, J.B.; Bruhn, J.N.; Kasimer, D.; Wang, H.; Rodrigue, N.; Smith, M.L. Clonal evolution and genome stability in a 2500-year-old fungal individual. *Proc. Biol. Sci.* **2018**, *285*, 20182233.

Roberts, S.E.; Gladfelter, A.S. Nuclear autonomy in multinucleate fungi. *Curr. Opin. Microbiol.* **2015**, *28*, 60–65.

Pieuchot, L.; Lai, J.; Loh, R.A.; Leong, F.Y.; Chiam, K.H.; Stajich, J.; Jedg, G. Cellular subcompartments through cytoplasmic streaming. *Dev. Cell.* **2015**, *34*, 410–420.

Lai, J.; Koh, C.H.; Tjota, M.; Pieuchot, L.; Raman, V.; Chandrababu, K.B.; Yang, D.; Wong, L.; Jedg, G. Intrinsically disordered proteins aggregate at fungal cell-to-cell channels and regulate intercellular connectivity. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 15781–15786.

Nitsche, B.M.; Burggraaf-van Welzen, A.M.; Lamers, G.; Meyer, V.; Ram, A.F. Autophagy promotes survival in aging submerged cultures of the filamentous fungus *Aspergillus niger*. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 8205–8218.

Anderson, C.A.; Roberts, S.; Zhang, H.; Kelly, C.M.; Kendall, A.; Lee, C.; Gerstenberger, J.; Koenig, A.B.; Kabeche, R.; Gladfelter, A.S. Plodiy variation in multinucleate cells changes under stress. *Mol. Biol. Cell* **2015**, *26*, 1129–1140.

Roper, M.; Lee, C.; Hickey, P.C.; Gladfelter, A.S. Life as a moving fluid: Fate of cytoplasmic macromolecules in dynamic fungal syncytia. *Curr. Opin. Microbiol.* **2015**, *26*, 116–122.

Zachetti, B.; Wosten, H.A.B.; Claessen, D. Multiscale heterogeneity in filamentous microbes. *Biotechnol. Adv.* **2018**, *36*, 2138–2149.

Teichert, I.; Wolff, G.; Kuck, U.; Nowroussian, M. Combining laser microdissection and RNA-seq to chart the transcriptional landscape of fungal development. *BMC Genom.* **2012**, *13*, 511.

de Bekker, C.; van Veluw, G.J.; Vinck, A.; Wiebenga, L.A.; Wosten, H.A. Heterogeneity of *Aspergillus niger* microcolonies in liquid shaken cultures. *Appl. Environ. Microbiol.* **2011**, *77*, 1263–1267.

Vinck, A.; Terlou, M.; Pestman, W.R.; Martens, E.P.; Ram, A.F.; van den Hondel, C.A.M.J.J.; Wosten, H.A.B. Hyphal differentiation in the exploring mycelium of *Aspergillus niger*. *Mol. Microbiol.* **2005**, *58*, 693–699.

Kasuga, T.; Glass, N.L. Dissecting colony development of *Neurospora crassa* using mRNA profiling and comparative genomics approaches. *Eukaryot. Cell* **2008**, *7*, 1549–1564.

Levin, A.M.; de Vries, R.P.; Conesa, A.; de Bekker, C.; Talon, M.; Menke, H.H.; van Peij, N.N.; Wosten, H.A. Spatial differentiation in the vegetative mycelium of *Aspergillus niger*. *Eukaryot. Cell* **2007**, *6*, 2311–2322.

Caten, C.E.; Jinks, J.L. Heterokaryosis: Its significance in wild homothallic ascomycetes and fungi imperfecti. *Trans. Br. Mycol. Soc.* **1966**, *49*, 81–93.

Strom, N.B.; Bushley, K.E. Two genomes are better than one: History, genetics, and biotechnological applications of fungal heterokaryons. *Fungal Biol. Biotechnol.* **2016**, *3*, 4.

Gehrmann, T.; Pelkmans, J.F.; Ohm, R.A.; Vos, A.M.; Sonnenberg, A.S.M.; Baars, J.J.P.; Wosten, H.A.B.; Reinders, M.J.T.; Abeel, T. Nucleus-specific expression in the multinuclear mushroom-forming fungus
Agaricus bisporus reveals different nuclear regulatory programs. Proc. Natl. Acad. Sci. USA 2018, 115, 4429–4434.

73. Kafer, E. An 8-chromosome map of Aspergillus nidulans. Adv. Genet. 1958, 9, 105–145.
74. McGuire, I.C.; Davis, J.E.; Double, M.L.; MacDonald, W.L.; Rauscher, J.T.; McCawley, S.; Milgroom, M.G. Heterokaryon formation and parasexual recombination between vegetatively incompatible lineages in a population of the chestnut blight fungus, Cryphonectria parasitica. Mol. Ecol. 2005, 14, 3657–3669.
75. Tsai, H.J.; Nellant, A. A double-edged sword: Aneuploidy is a prevalent strategy in fungal adaptation. Genes 2019, 10, 787.
76. Miao, V.P.; Covert, S.F.; VanEtten, H.D. A fungal gene for antibiotic resistance on a dispensable (“B”) chromosome. Science 1991, 254, 1773–1776.
77. Ma, L.J.; van der Does, H.C.; Borkovich, K.A.; Coleman, J.J.; Daboussi, M.J.; Di Pietro, A.; Dufresne, M.; Freitag, M.; Grabherr, M.; Henriessat, B., et al. Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. Nature 2010, 464, 367–373.
78. Rep, M.; Kistler, H.C. The genomic organization of plant pathogenicity in Fusarium species. Curr. Opin. Plant Biol. 2010, 13, 420–426.
79. Shahi, S.; Beerens, B.; Bosch, M.; Limmans, J.; Rep, M. Nuclear dynamics and genetic rearrangement in heterokaryotic colonies of Fusarium oxysporum. Fungal Genet. Biol. 2016, 91, 20–31.
80. Zakharov, I.A.; Yarovsky, B.P. Cytoduction as a new tool in studying the cytoplasmic heredity in yeast. Mol. Cell. Biochem. 1977, 14, 15–18.
81. Mela, A.P.; Momany, M. Internuclear diffusion of histone H1 within cellular compartments of Aspergillus nidulans. PLoS ONE 2018, 13, e0201828.
82. Roca, M.G.; Kuo, H.C.; Lichius, A.; Freitag, M.; Read, N.D. Nuclear dynamics, mitosis, and the cytoskeleton during the early stages of colony initiation in Neurospora crassa. Eukaryot. Cell 2010, 9, 1171–1183.
83. Jinks, J.L. Heterokaryosis; a system of adaption in wild fungi. Proc. R. Soc. Lond. B Biol. Sci. 1952, 140, 83–99.
84. Davis, R.H. Adaptation in pantothenate-requiring Neurospora. II. Nuclear competition during adaptation. Am. J. Bot. 1960, 47, 648–654.
85. James, T.Y.; Stenlid, J.; Olson, A.; Johannesson, H. Evolutionary significance of imbalanced nuclear ratios within heterokaryons of the basidiomycete fungus Heterobasidion parviporum. Evolution 2008, 62, 2279–2296.
86. Ramsdale, M.; Rayner, A.D.M. Imbalanced nuclear ratios, post-germination mortality and phenotype-genotype relationships in allopatrically-derived heterokaryons of Heterobasidion annosum. New Phytol. 1996, 133, 303–319.
87. James, T.Y.; Johannsson, S.B.; Johannesson, H. Trikaryon formation and nuclear selection in pairings between heterokaryons and homokaryons of the root rot pathogen Heterobasidion parviporum. Mycol. Res. 2009, 113, 583–590.
88. Zeyl, C.; DeVisscher, J.A. Estimates of the rate and distribution of fitness effects of spontaneous mutation in Saccharomyces cerevisiae. Genetics 2001, 157, 53–61.
89. Bastiaans, E.; Debets, A.J.; Aanen, D.K. Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters. Nat. Commun. 2016, 7, 11435.
90. Buss, L.W. Somatic cell parasitism and the evolution of somatic tissue compatibility. Proc. Natl. Acad. Sci. USA 1982, 79, 5337–5341.
91. Samils, N.; Oliva, J.; Johannesson, H. Nuclear interactions in a heterokaryon: Insight from the model Neurospora tetrasperma. Proc. Biol. Sci. 2014, 281. doi:10.1098/rspb.2014.0084.
92. Freitag, M.; Hickey, P.C.; Raju, N.B.; Selker, E.U.; Read, N.D. GFP as a tool to analyze the organization, dynamics and function of nuclei and microtubules in Neurospora crassa. Fungal Genet. Biol. 2004, 41, 897–910.
93. Gladfelter, A.S. Nuclear anarchy: Asynchronous mitosis in multinucleated fungal hyphae. Curr. Opin. Microbiol. 2006, 9, 547–552.
94. Rosenberger, R.F.; Kessel, M. Synchrony of nuclear replication in individual hyphae of Aspergillus nidulans. J. Bacteriol. 1967, 94, 1464–1469.
95. Clutterbuck, A.J. Synchronous nuclear division and septation in Aspergillus nidulans. J. Gen. Microbiol. 1970, 60, 133–135.
96. Anderson, C.A.; Eser, U.; Korndorf, T.; Borsuk, M.E.; Skotheim, J.M.; Gladfelter, A.S. Nuclear repulsion enables division autonomy in a single cytoplasm. Curr. Biol. 2013, 23, 1999–2010.
han, T.W.; Kato, M.; Xie, S.; Wu, L.C.; Mirzaei, H.; Pei, J.; Chen, M.; Xie, Y.; Allen, J.; Xiao, G., et al. Cell-free formation of RNA granules: Bound RNAs identify features and components of cellular assemblies. Cell 2012, 149, 768–779.

98. Kato, M.; Han, T.W.; Xie, S.; Shi, K.; Du, X.; Wu, L.C.; Mirzaei, H.; Goldsmith, E.J.; Longgood, J.; Pei, J., et al. Cell-free formation of RNA granules: Low complexity sequence domains form dynamic fibers within hydrogels. Cell 2012, 149, 753–767.

99. Li, P.; Banjade, S.; Cheng, H.C.; Kim, S.; Chen, B.; Guo, L.; Llaguno, M.; Hollingsworth, J.V.; King, D.S.; Banani, S.F., et al. Phase transitions in the assembly of multivalent signalling proteins. Nature 2012, 483, 336–340.

100. Lee, C.; Occhipinti, P.; Gladfelter, A.S. PolyQ-dependent RNA-protein assemblies control symmetry breaking. J. Cell Biol. 2015, 208, 533–544.

101. Lee, C.; Zhang, H.; Baker, A.E.; Occhipinti, P.; Borsuk, M.E.; Gladfelter, A.S. Protein aggregation behavior regulates cyclin transcript localization and cell-cycle control. Dev. Cell 2013, 25, 572–584.

102. Dundon, S.E.; Chang, S.S.; Kumar, A.; Occhipinti, P.; Shroff, H.; Roper, M.; Gladfelter, A.S. Clustered nuclear maintain autonomy and nucleocytoplasmic ratio control in a syncytium. Mol. Biol. Cell 2016, 27, 2000–2007.

103. Langdon, E.M.; Qiu, Y.; Ghanbari Niaki, A.; McLaughlin, G.A.; Weidmann, C.A.; Gerbic, T.M.; Smith, J.A.; Crutchley, J.M.; Termini, C.M.; Weeks, K.M., et al. mRNA structure determines specificity of a polyQ-driven phase separation. Science 2018, 360, 922–927.

104. Plamann, M.; Minke, P.F.; Tinsley, J.H.; Bruno, K.S. Cytoplasmic dynein and actin-related protein Arp1 are required for normal nuclear distribution in filamentous fungi. J. Cell Biol. 1994, 127, 139–149.

105. Xiang, X.; Beckwith, S.M.; Morris, N.R. Cytoplasmic dynein is involved in nuclear migration in Aspergillus nidulans. Proc. Natl. Acad. Sci. USA 1994, 91, 2100–2104.

106. Alberti-Segui, C.; Dietrich, F.; Altman-Johl, R.; Hoepfner, D.; Philippsen, P. Cytoplasmic dynein is required to oppose the force that moves nuclei towards the hyphal tip in the filamentous ascomycete Ashbya gossypii. J. Cell Sci. 2001, 114, 975–986.

107. Sachsenmaier, W.; Remy, U.; Plattner-Schobel, R. Initiation of synchronous mitosis in Physarum polycephalum. A model of the control of cell division in eukariots. Exp. Cell Res. 1972, 73, 41–48.

108. Dynesen, J.; Nielsen, J. Branching is coordinated with mitosis in growing hyphae of Aspergillus nidulans. Fungal Genet. Biol. 2003, 40, 15–24.

109. Neumann, F.R.; Nurse, P. Nuclear size control in fission yeast. J. Cell Biol. 2007, 179, 593–600.

110. Kume, K.; Cantwell, H.; Neumann, F.R.; Jones, A.W.; Snijders, A.P.; Nurse, P. A systematic genomic screen implicates nucleocytoplasmic transport and membrane growth in nuclear size control. PLoS Genet. 2017, 13, e1006767.

111. Roberts, S.E.; Gladfelter, A.S. Nuclear dynamics and cell growth in fungi. In Growth, Differentiation and Sexuality; Wendland, J., Ed.; Springer: Cham, Switzerland, 2016; pp. 27–46

112. May, G.; Taylor, J.W. Patterns of mating and mitochondrial DNA inheritance in the agaric Basidiomycete Coprinus cinereus. Genetics 1988, 118, 213–220.

113. Lee, S.B.; Taylor, J.W. Uniparental inheritance and replacement of mitochondrial DNA in Neurospora tetrasperma. Genetics 1993, 134, 1063–1075.

114. Daubois, L.; Beaudet, D.; Hjiri, M.; de la Providencia, I. Independent mitochondrial and nuclear exchanges arising in Rhizopus irregularis crossed-isolates support the presence of a mitochondrial segregation mechanism. BMC Microbiol. 2016, 16, 11.

115. Aanen, D.K.; Kuyper, T.W.; Debets, A.J.; Hoekstra, R.F. The evolution of non-reciprocal nuclear exchange in mushrooms as a consequence of genomic conflict. Proc. Biol. Sci. 2004, 271, 1235–1241.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).