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REVIEW

Fungal Morphogenesis, from the Polarized Growth of Hyphae to Complex Reproduction and Infection Structures

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SUMMARY Filamentous fungi constitute a large group of eukaryotic microorganisms that grow by forming simple tube-like hyphae that are capable of differentiating into more-complex morphological structures and distinct cell types. Hyphae form filamentous networks by extending at their tips while branching in subapical regions. Rapid tip elongation requires massive membrane insertion and extension of

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the rigid chitin-containing cell wall. This process is sustained by a continuous flow of secretory vesicles that depends on the coordinated action of the microtubule and actin cytoskeletons and the corresponding motors and associated proteins. Vesicles transport cell wall-synthesizing enzymes and accumulate in a special structure, the Spitzenkörper, before traveling further and fusing with the tip membrane. The place of vesicle fusion and growth direction are enabled and defined by the position of the Spitzenkörper, the so-called cell end markers, and other proteins involved in the exocytic process. Also important for tip extension is membrane recycling by endocytosis via early endosomes, which function as multipurpose transport vehicles for mRNA, septins, ribosomes, and peroxisomes. Cell integrity, hyphal branching, and morphogenesis are all processes that are largely dependent on vesicle and cytoskeleton dynamics. When hyphae differentiate structures for asexual or sexual reproduction or to mediate interspecies interactions, the hyphal basic cellular machinery may be reprogrammed through the synthesis of new proteins and/or the modification of protein activity. Although some transcriptional networks involved in such reprogramming of hyphae are well studied in several model filamentous fungi, clear connections between these networks and known determinants of hyphal morphogenesis are yet to be established.

KEYWORDS hyphal morphogenesis, fungal development, polarity, cytoskeleton, cell wall

INTRODUCTION

Fungi, ranging from single-cell yeasts to some of the largest organisms on earth, are eukaryotes with a long tradition in fundamental research. Numerous groundbreaking discoveries have been made in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, such as the understanding of the cell cycle by Leland Hartwell, Tim Hunt, and Paul Nurse (Nobel Prize for Physiology or Medicine in 2001). In addition to these yeast-based discoveries, research focused on filamentous fungi has resulted in major breakthroughs. *Neurospora crassa* was the organism with which Edward Tatum and George Beadle developed the "one gene, one enzyme" concept (Nobel Prize for Physiology or Medicine in 1958). Gamma-tubulin was discovered by Berl Oakley in *Aspergillus nidulans* in 1989 and was found in all eukaryotes afterwards. Other examples where fungi contributed to our basic understanding of eukaryotic cell function include cell wall biosynthesis, circadian clock function, gene silencing, and DNA repair and recombination models.

Besides their importance in fundamental research, fungi are extremely important for organic material recycling in nature. Fungi have also been used as food sources and for food processing for thousands of years, and fungi are important workhorses in modern biotechnology. Furthermore, pathogenic fungi cause huge losses in agriculture and can be life threatening to humans (1). Fungi destroy more than 125 million tons of the top five food crops (rice, wheat, maize, potatoes, and soybeans) every year (2). Food contamination by mycotoxins results in estimated losses of over US$1.5 billion annually (3). Of all known human pathogens, about 20% (325 species) are fungi (4). Hence, a better understanding of the fungal lifestyle, cell biology, metabolism, and morphogenesis can have a strong impact on our daily lives. Many basic properties are conserved among model yeasts, such as *S. cerevisiae* and *S. pombe*, and filamentous fungi. However, properties such as a rich secondary metabolism and the ability to undergo complex multicellular development or to form specialized infection structures are exclusive to filamentous fungi. Even basic processes, such as polar cell extension, may be different in different fungal species when analyzed at molecular and ultrastructural levels. Therefore, a better understanding of fungal biology requires comparative studies of different fungi. Such comparative studies are becoming feasible thanks to the progress in efficient gene deletion methods and the use of different fluorescent proteins in combination with high-end microscopy. So far, the clearest pictures have come from a few well-studied filamentous fungi.

This review summarizes the cellular and molecular basis for hyphal morphogenesis and development by comparing what is known about the fungi *Neurospora crassa*, *Aspergillus nidulans*, *Sordaria macrospora*, *Trichoderma atroviride*, and *Ustilago maydis*. Development in filamentous fungi involves drastic changes in growth patterns that finally result in shape determination. The sustained polarized growth of hyphae is slowed or completely halted to give rise to different developmental structures, such as various types of conidiophores and conidia; different fruiting bodies and sexual spores; specialized hyphae, like clamp connections; or infection structures, such as appressoria or infection pegs. Recent findings about cell signaling and the reprogramming of gene and protein expression patterns that occur during these processes are discussed.

THE FUNGAL HYPHA

Hyphal Shape and Cell Wall Synthesis

The vegetative unit of growth in filamentous fungi is the hypha, a cylindrical cell with a characteristic tip whose shape approximates that of a hemiellipsoid but can be more precisely defined mathematically by the hyphoid equation (5). This accurate description of shape is not just a theoretical exercise but also a handle for understanding how morphology is generated in the region where growth is concentrated. Besides being the most widespread form of growth in fungi, the filamentous form seems to be a more ancestral mode of growth than the yeast form (6). Fungal hyphae extend by tip growth (7, 8), in a process that encompasses the polarized transport of vesicles to growth sites, where they fuse to ensure the localized deposition of new plasma membrane and cell wall material (9). Tip growth and hyphal morphology in most filamentous fungi belonging to the dikarya depend on the Spitzenkörper, an apical body composed of vesicles, actin, ribosomes, and an amorphous material of an unidentified nature (10–13). Hyphae of most zygomycetous fungi and other early fungal lineages do not present a typical Spitzenkörper (14). Instead, most of them display a simpler apical vesicle crescent (AVC) beneath the apical plasma membrane of growing hyphae, which is believed to function as the Spitzenkörper (15, 16).

Hyphal shape is constructed by a polarized gradient of cell wall deposition (5). In the cell wall of most fungi, interwoven microfibrils of chitin and β-1,3-glucans are embedded in an amorphous gel-like matrix composed of polysaccharides (α-1,3-glucans) and glycoproteins (mainly galactomannoproteins). Chitin synthases (CHSs) constitute a family of membrane-embedded enzymes that catalyze the synthesis of chitin at sites of cell wall expansion. The genomes of filamentous fungi encode up to seven different classes of CHSs, in contrast to up to three classes of CHSs present in yeast or dimorphic species (17). β-1,3-Glucans are synthesized by a β-1,3-glucan synthase complex (GSC), with catalytic (Fks) and regulatory (Rho1) subunits. Filamentous fungi contain only one essential *fks* gene, while two *FKS* genes and as many as four *BGS* genes have been identified in *S. cerevisiae* and *S. pombe*, respectively. In *S. cerevisiae*, *FKS1* and *FKS2* have overlapping functions. In *S. pombe*, *BGS1*, *BGS2*, *BGS3*, and *BGS4* have distinct activities but seem to share some overlapping roles as well. Some authors suggested a link between the higher capacity to produce β-1,3-glucan of some fungal phyla and tolerance to drought stress (18). Along with this argument, yeasts are considered "tolerators" and have traits associated with stress resistance, whereas filamentous fungi are "competitors."

CHS and the GSC are transported in an inactive form within vesicles that fuse with the plasma membrane, where the enzymes are inserted to synthesize *in situ* the chitin and β-1,3-glucan microfibrils, the polysaccharides that constitute the skeletal fraction of the wall. Glycoproteins are presynthesized and transported through the secretory pathway and, after exocytosis, remain attached to the plasma membrane through a glycosylphosphatidylinositol (GPI) anchor.

It is not fully understood how a rigid structure such as the cell wall expands or suffers changes during tip elongation, branch emergence, and the development of reproductive structures, etc. Proposed models have tried to explain this process of cell wall remodeling at the hyphal tip. The unitary model of cell wall growth suggested a

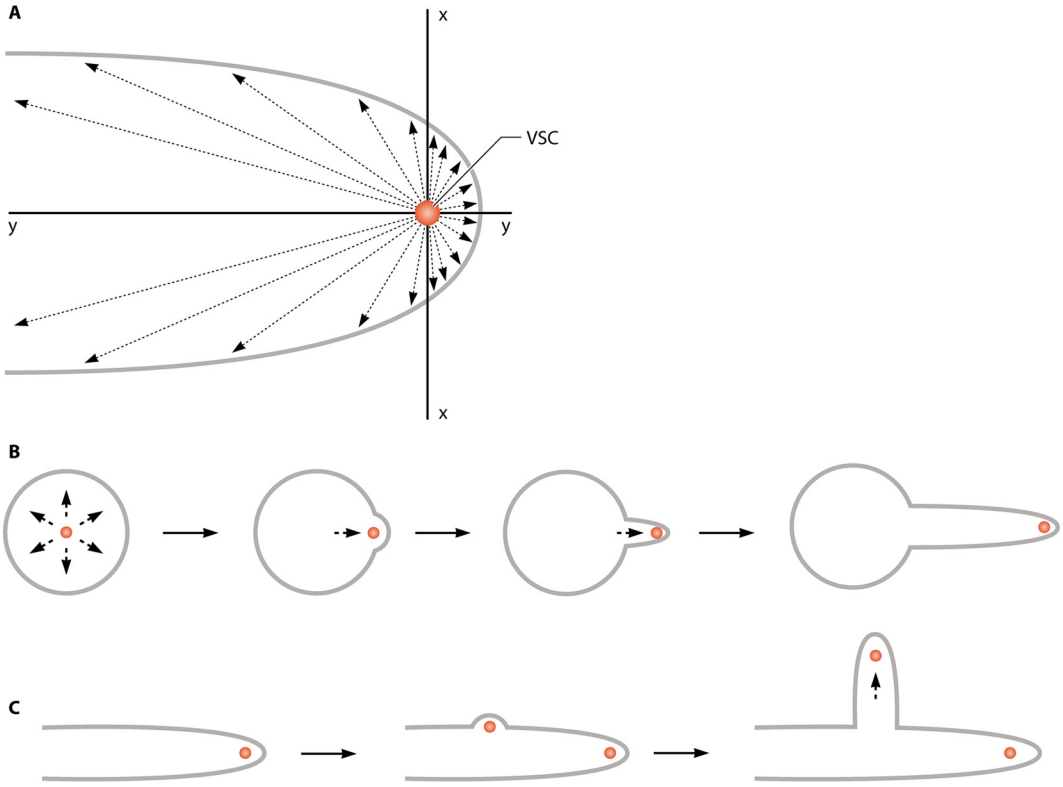

FIG 1 VSC (vesicle supply center) model for hyphal morphogenesis. (A) The "hyphoid," a perfect hyphal shape. The hyphoid curve is a geometric function derived from a computer-simulated secretory process, where growth units (vesicles) emanating from a forward-moving source (the VSC) extend the cell surface in a sharply polarized manner. When analyzed mathematically, the process yielded the hyphoid equation $y = x \cot (x\,V/N)$, where N is the amount of cell wall-building vesicles produced per unit of time and V is the rate of advancement of the VSC; when plotted on Cartesian coordinates, the function generates a unique curve that follows closely the actual profile of regular hyphae (Adapted from reference 23.) (B) Displacement and advancement of the VSC from its concentric position in a spore generate a germinating tube. (C) The formation and advancement of a new VSC at a subapical hyphal region generate a lateral branch.

simultaneous and balanced action of cell wall-loosening enzymes and cell wall-synthesizing enzymes at hyphal apices (19). The steady-state model proposed that the apical cell wall is plastic and therefore expandable and becomes rigid in the subapex by the action of cross-linking enzymes (20). Corroborating evidence, such as the localization of cross-linking enzymes at the subapex, has not been attained so far. In *N. crassa*, the putative β-1,3-endoglucanases BGT-1 and BGT-2 were found at the hyphal apical plasma membrane immediately behind the apical pole (21). In *A. nidulans*, the chitinase ChiA was also localized at the apical plasma membrane of germ tubes, hyphae, and branches (22). The most accepted current view proposes that cell wall-loosening enzymes, such as chitinases and glucanases, participate in the breakage of polysaccharide chains, such as chitin and β-1,3-glucans, on the one hand allowing the incorporation of the newly deposited material and on the other hand generating free ends, which would serve as the substrate for cross-linking enzymes that would rigidify the cell wall.

A vesicle supply center (VSC) in motion provides a rational basis to predict how the secretory apparatus generates shape, i.e., morphogenesis. Advancing the VSC forward in a linear fashion while at the same time releasing vesicles would produce an ideal hypha (Fig. 1). The Spitzenkörper is believed to function as a VSC that regulates the delivery of cell wall-building vesicles to the apical cell surface (23). By programming a VSC to advance as a Spitzenkörper, it was possible to mimic the hyphal growth of *N. crassa* wild-type (WT) and mutant strains by computer simulation (24, 25). Similarly, manipulation of the position, speed, or behavior of the VSC could simulate other cell types or developmental stages. For instance, simulation of branch emergence with the

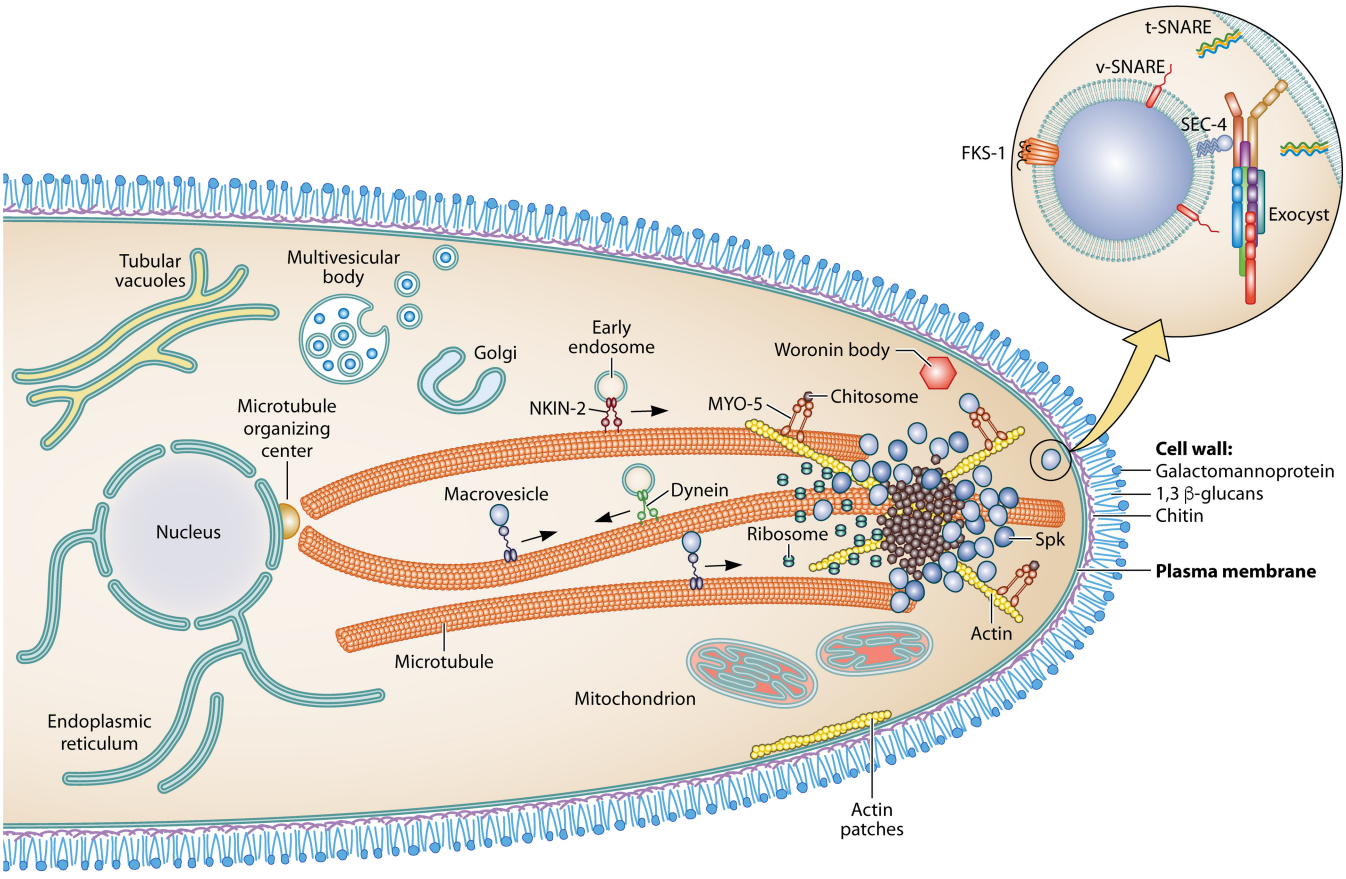

FIG 2 Architecture of a fungal hyphal tip. Different populations of vesicles concentrate at the Spitzenkörper. Examples of the main organelles of the secretory pathway and the cytoskeleton are displayed. The circle shows a macrovesicle carrying β-1,3-glucan synthase and the protein complexes required for vesicle fusion with the plasma membrane.

VSC model would imply the formation of a new VSC, whereas germination could be achieved simply by displacing the preexisting VSC from the center to the periphery of a conidium (Fig. 1).

The Secretory Pathway

Evidence from diverse sources indicates that the Spitzenkörper determines hyphal morphology and growth direction (24–28).

In *N. crassa*, the localization and dynamics of the main cell wall-synthesizing enzymes have been elucidated (29–32). All CHSs localize at the Spitzenkörper core, whereas macrovesicles carrying the GSC occupy the Spitzenkörper outer layer (Fig. 2). This distinct functional stratification at the Spitzenkörper parallels the vesicular arrangement identified by transmission electron microscopy (TEM), with a core of chitosomes (a population of specialized microvesicles with CHS activity) surrounded by an array of macrovesicles. While some CHSs in *A. nidulans* have been localized at the Spitzenkörper (33), it has not been revealed whether they are distributed at the Spitzenkörper core or outer layer. However, biochemical stratification in the Spitzenkörper was observed in *A. nidulans*, with the spatial distribution of DnfA and DnfB, two P4 ATPases (34).

The biogenesis and transport of the vesicular carriers that accumulate at the Spitzenkörper prior to exocytosis are still poorly understood. As in other eukaryotes, vesicle biogenesis and delivery comprise basic steps, including vesicle budding from the donor membrane, transport, docking to a target membrane, and vesicle fusion (35). All these steps require the participation of coat complexes, tethers, Rab GTPases, and SNARE (soluble NSF [*N*-ethylmaleimide-sensitive factor] attachment protein [SNAP] receptor).

In *A. nidulans*, traffic regulators of secretion, RabC^{Rab6}, RabO^{Rab1}, RabD^{Rab8}, and RabE^{Rab11}, were detected in the Spitzenkörper (36–38). In *N. crassa*, the corresponding orthologues of these Rab GTPases occupy distinctly the two layers of the Spitzenkörper; YPT-1^{Rab1} was found in the core, while SEC-4^{Rab8} and YPT-31^{Rab11} were located in the outer layer, suggesting that distinct Rabs regulate the traffic of the different vesicle populations to the Spitzenkörper (39). Quantitative analyses based on FRAP (fluorescence recovery after photobleaching) experiments identified vesicle turnover rates at the Spitzenkörper ranging from 20 to 40 s; i.e., in less than a minute, a full set of vesicles arrives at and departs from the Spitzenkörper (39). It has been proposed that the Rab-associated vesicular carriers delivered to the Spitzenkörper are derived from the Golgi apparatus (38). In filamentous fungi, as in yeast, the Golgi apparatus is a collection of early (*cis*) and late (*trans*) cisternae. In both *A. nidulans* and *N. crassa*, early Golgi cisternae (EGC) and late Golgi cisternae (LGC) are distributed along the hyphae displaying a polarized gradient from EGC to LGC but are excluded from the apical region (39, 40). This distribution would support the widely accepted Golgi cisternal maturation model explaining the transit of lipids and proteins through the Golgi apparatus and their sorting into carriers directed to the plasma membrane or to endosomes (41). In *A. nidulans*, a multisubunit tethering complex, the "transport particle protein" TRAPPII, best analyzed in *S. cerevisiae*, has been studied. TRAPPII participates in intra-Golgi, endosome-to-Golgi, and Golgi exit trafficking through the GTP exchange of Rab GTPases. In *A. nidulans*, the HypA (Trs120) subunit of TRAPPII has been shown to participate in Golgi-to-post-Golgi traffic (42–44).

Upon arrival to the immediacies of the apical plasma membrane, vesicles are tethered via the exocyst (Fig. 2), a conserved octameric tethering complex first discovered in yeast. Exocyst components have been found in filamentous fungi at growth sites (45, 46). In the larger hyphae of *N. crassa*, the subcellular organization of the apex can be more distinctly resolved by confocal laser scanning microscopy. Some of the exocyst components accumulate adjacent to the apical plasma membrane, while others (EXO-70 and EXO-84) localize at the most proximal part of the Spitzenkörper outer layer (45). This suggests that exocyst components attach to the macrovesicles that are ready to depart from the Spitzenkörper and go on to fuse with the apical plasma membrane. Whether the exocytosis of the microvesicles requires a tether-dependent mechanism remains to be determined. For fungi other than yeast, exocyst knowledge is rather scarce.

After exocyst tethering, vesicles fuse with their target membrane in a process mediated by SNARE interactions (Fig. 2). In *A. nidulans*, the synaptobrevin vesicular SNARE protein SynA (orthologue of yeast Snc1p) tagged with green fluorescent protein (GFP) was observed at the Spitzenkörper and at the apical plasma membrane (47). In *N. crassa*, GFP–SYN-1 was observed at the Spitzenkörper but not at the plasma membrane (39).

Endocytosis and Hyphal Morphogenesis

As mentioned above, hyphal elongation implies the continuous addition of new plasma membrane, proteins, and cell wall material at the hyphal apex (9). Recent theoretical calculations and FRAP measurements of endocytic events indicate that the amount of membrane transferred by exocytosis is in excess of that needed for plasma membrane extension (48), hence the need for endocytosis, a mechanism that deals with this excess of membrane and in turn allows the reutilization or the degradation of membrane proteins (49–54). In the hyphal apex and subapex, there is a spatial proximity between the exocytosis and endocytosis regions, supporting the idea that both processes seem to operate in tandem as part of the polarized machinery responsible for hyphal morphogenesis (55–58).

In fungal hyphae, most endocytosis takes place at a short distance from the apex in a rather-well-defined region of the subapex named the endocytic collar, first described in *A. nidulans* (55, 56) and also described in *N. crassa* (57). In *A. nidulans*, the deletion of fimbrin (a protein which cross-links actin) caused severe defects in polar growth, providing evidence for an important role of actin patches and endocytosis for polar

growth (55, 56). Analysis of deletion mutants of *N. crassa* lacking coronin (Δ*crn-1*) (58) or myosin-1 (Δ*myo-1*) (59), both of which are components of the endocytic machinery, gave further insight into the role of endocytosis in hyphal morphogenesis. In the Δ*crn-1* as well as the Δ*myo-1* mutants, hyphal morphology and growth directionality were disturbed, generating intermittent periods of polarized and isotropic growth. These alterations could be attributed to disorders of the pattern of vesicle migration to the apex, thus preventing the formation of a normal hypha with a regular shape (9, 23). Although the impact of endocytosis on apical exocytosis has not been addressed, the link between exocytosis and endocytosis appears straightforward, with endocytosis being the consequence of the excess accumulation of plasma membrane discharged by exocytosis.

Positional Information for Growth: Cell End Markers, the Polarisome, and Motors

A number of proteins involved in polarized growth are transported along microtubules (MTs) and the actin cytoskeleton to the hyphal tips (38, 47, 60). Microtubules serve as tracks for secretory vesicles for long-distance transport to the hyphal tip and are important for rapid hyphal growth (61). Actin cables, formed from the hyphal tip in the retrograde direction, are involved in exocytosis and secretory vesicle accumulation at the Spitzenkörper, prior to exocytosis (62, 63). Vesicles containing components of the tip growth machinery are thought to be transported along microtubules from posterior sites to the apical region, transferred to actin cables, and delivered to the apical cortex of the hypha (38, 64–67).

Besides their role as tracks for vesicle traffic, microtubules are necessary to maintain the growth direction of hyphae (25). The polar organization of the actin cytoskeleton is mediated mainly by the microtubule-dependent positioning of proteins known as cell end markers, initially discovered in the fission yeast *S. pombe* (64, 68–70). TeaA, one cell end marker in *A. nidulans*, is specifically delivered to the apex by growing microtubules and is anchored at the membrane by direct interactions with TeaR, another cell end marker at the plasma membrane (71) (Fig. 3). The interaction of TeaA and TeaR at the apical membrane initiates the recruitment of additional downstream components, including the formin SepA, which polymerizes actin cables for targeted cargo delivery (72). Defective cell end marker proteins result in highly curved or meandering hyphae instead of the straight wild-type form (71) (Fig. 3). Within the growth region of the cell, the position of the polarity site may shift to change the direction of growth, but the polarity site has hitherto been viewed as a persistent feature of the growth region of the cell. This mechanism is also required the for asexual development and pathogenesis of *Magnaporthe oryzae* (73). The orthologous genes are conserved in other Sordariomycetes and Basidiomycota (66); however, the exact role needs to be determined.

For many decades, there was no clear explanation as to how cell polarity is maintained during continuous vesicle exocytosis, especially for rapidly growing systems such as those of filamentous fungi. In fact, in *A. nidulans*, the rate of elongation is 10 to 30 times higher than that of bud formation in budding yeast, a rate at which polarity determinants are predicted to become disturbed by incoming vesicles (61, 74, 75). The cell extension rate of *N. crassa* is up to 10 times higher than the one of *A. nidulans* (76). Still, filamentous fungi are able to maintain cell polarity in the presence of thousands to several tens of thousands of exocytic events predicted to occur at the cell tip (apex) every minute (77, 78).

The resolution of conventional light microscopy techniques is limited to around 250 to 300 nm due to light diffraction. Superresolution microscopy techniques, such as stimulated emission depletion microscopy (STED), structured illumination microscopy (SIM), stochastic optical reconstruction microscopy (STORM), and photoactivation localization microscopy (PALM), etc., have overcome the diffraction limit, resulting in lateral image resolution as high as 20 nm, providing a powerful tool to investigate protein localization in high detail (79, 80). Recent studies using PALM revealed a dynamic picture of the membrane-associated TeaR polarity marker in *A. nidulans* (77) (Fig. 3). PALM analyses clearly showed TeaR clusters near the apex of the cell, along the plasma membrane, with average sizes of approximately 120 nm. It was estimated that

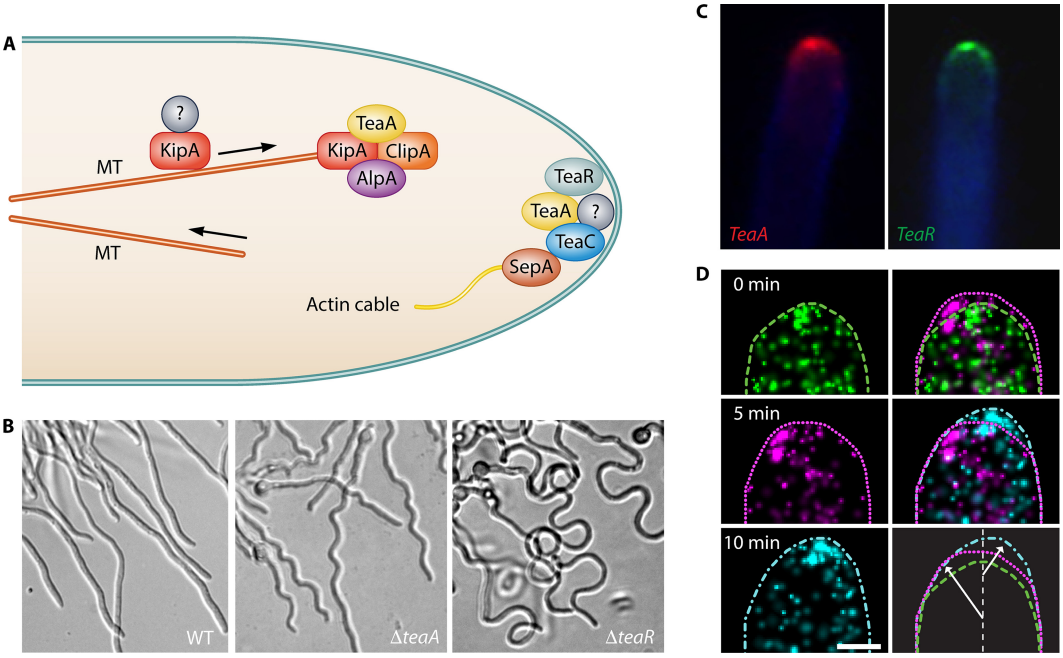

FIG 3 Cell end marker proteins determine the interplay between the microtubule and the actin cytoskeleton in *A. nidulans*. (A) Scheme of cell end markers transported at the MT plus end and delivered to the apical membrane. The prenylated TeaR proteins are probably delivered with vesicles. The motor protein KipA transports TeaA and probably other tip proteins toward the MT plus end. (B) Differential interference contrast images of wild-type, Δ*teaA*, and Δ*teaR* strains. Δ*teaA* strains exhibited zigzag and Δ*teaR* strains curved hyphae. (C) Monomeric red fluorescent protein 1 (mRFP1)-TeaA or GFP-TeaR localizes to one point at the tip and along the tip membrane. (D) Series of PALM images of an mEosFP-TeaR-expressing hypha (5-min time interval). Cell profiles are shown in different line styles. The right column shows overlays of PALM images from two time points (top, 0 and 5 min; middle, 5 and 10 min), and overlays of outlines reveal growth regions coinciding with TeaR cluster locations. (Panels A to C are modified from reference 64 with permission; panel D is modified from reference 77.)

about 20 TeaR proteins composed each of these clusters. Time-lapse PALM revealed transient assemblies of TeaR at the membrane of hyphal tips and tethering of vesicles there. These assemblies dispersed along the membrane after exocytosis, indicating an exocytic mechanism that inserts new membrane and results in local membrane extension. This observation suggested a "transient polarity assembly model," which explains that fungal tip cells extend by a repetition of coordinated steps, TeaR assembly/disassembly, actin polymerization, and exocytosis, rather than by constant elongation.

Oscillation rates of hyphal extension were reported for several filamentous fungi more than 20 years ago (81), but the cellular or molecular mechanisms behind hyphal extension remained unclear. Oscillations of Ca^{2+} levels have been observed at hyphal tips (82), and Ca^{2+} levels have been proven to regulate actin assembly and vesicle fusion (83, 84). This strongly suggests that the oscillatory rate of extension of hyphal tips involves a concerted action of Ca^{2+}, actin, and exocytosis. In fact, live-cell imaging analysis, including superresolution microscopy, revealed that the *A. nidulans* hyphal tip extends in an oscillatory manner (76, 85). The amounts of F-actin and secretory vesicles accumulating at the hyphal tip oscillated with a positive temporal correlation. Intracellular Ca^{2+} levels also pulsed with a positive temporal correlation to the amounts of F-actin and secretory vesicles at the hyphal tip. Ca^{2+} channels were needed for proper tip growth and oscillations of actin polymerization, exocytosis, and the growth rate. These data support a model in which temporally controlled actin polymerization and exocytosis are coordinated by pulsed Ca^{2+} influx, resulting in oscillatory cell extension.

Homologues of the Rho-type GTPases Cdc42 and Rac1 have been studied in *A. nidulans* (ModA and RacA), *N. crassa* (CDC-42 and RAC-1), and *U. maydis* (Cdc42 and Rac1) (86–88). Both Cdc42 and Rac1 share at least one overlapping function that is required for polarity establishment. The combination of Δ*cdc42* with Δ*rac1* appeared synthetically lethal in *A. nidulans*. Cdc42, Rac1, and TeaR are directly anchored the

membrane through posttranslational lipid modification at the C-terminal CaaX motif. In *N. crassa*, the spatial distribution of these Rho GTPases changes from early to late differentiation stages. Prior to symmetry breakage in the conidia, the accumulation and localized activation of Cdc42 and its guanidine exchange factor (GEF) Cdc24 occur. After the emergence of a polarized germ tube, Rac is recruited at the incipient tip, forming a crescent. Cdc42 and Rac regulate the negative chemotropism exhibited during germ tube development and the positive chemotropism observed during conidial anastomosis tube (CAT) formation and cell fusion. In mature hyphae, Cdc42, Cdc24, and Rac are localized at the apical dome.

Polarisome components act downstream of Cdc42 and are conserved from yeast to filamentous fungi (89). In yeast, the main components of the polarisome are Spa2, Pea2, Aip3/Bud6, and the key effector Bni1 (90, 91). Through the formin Bni1, the polarisome in *S. cerevisiae* is involved in polarization by directing the localized assembly of actin filaments (92). The role of the polarisome components BudA and SpaA in *A. nidulans*, corresponding to *S. cerevisiae* Bud6 and Spa2, was shown to be dispensable for Spitzenkörper organization (93, 94). In *N. crassa*, BUD-6 and SPA-2 are required for the maintenance of apical growth and cell morphology in young germlings and mature hyphae (95, 96). SPA-2 accumulates at hyphal apices, colocalizing partially with the Spitzenkörper core. BUD-6 accumulates at the apical plasma membrane, excluding the very tip. In addition, the formin BNI-1, another putative polarisome component, has a distribution similar to that of BUD-6, but SPA-2 colocalizes with the Spitzenkörper. *Ashbya gossypii* Spa2 localizes to polarized growth sites and is involved in hyphal growth speed and guidance (97, 98). Interestingly, the localization of exocyst and polarisome components at the Spitzenkörper or the apical plasma membrane is correlated with growth speed in *A. gossypii* (99).

Besides protein complexes, sterol-rich membrane domains (SRDs), characterized by a high sterol content, play important roles in hyphal tip growth (100), where they were visualized by using the sterol-binding fluorescent dye filipin. Sterols and sphingolipids can cluster into domains within mixtures of glycerophospholipids. These domains, termed "lipid rafts," contribute to the specific localization of proteins, such as GPI-anchored and lipid-associated proteins, that play important roles in cell signaling and cell polarity (101–103). Filipin stains the tips of mating projections in *S. cerevisiae* (104), cell ends in *S. pombe* (105), germling tips in *N. crassa* (447), and hyphal tips in *Candida albicans* (106) and *A. nidulans* (107). In *S. pombe*, reentry into the cell cycle involves the *de novo* definition of growth zones via the reorganization of the SRD to the cell ends, accompanied by the recruitment of the cell growth machinery. As SRDs are specific to fungi and have not been found in mammalian cells, they might play fungus-specific roles in membrane organization and be considered potential antifungal drug targets.

THE FUNGAL CYTOSKELETON

The Microtubule Cytoskeleton

Microtubules (MTs) are hollow, normally unbranched rigid tubes arranged in parallel or forming an interwoven mesh within interphase hyphae (Fig. 4). One important property of MTs is their dynamic instability. They constantly elongate for some time, at most until they reach the cortex, and then depolymerize, at most to their origin, until they recover and grow again. Microtubules are involved in the transport and distribution of organelles from nuclei to vesicles and in the transport of proteins. There is some evidence that not all interphase MTs are identical (108–110). Posttranslational tubulin modifications are well known, and their functions are slowly becoming clearer (111). Both α- and β-tubulins can be substrates for modifications. One well-characterized modification of α-tubulin is the cleavage of a C-terminal tyrosine residue and thereby the exposure of glutamic acid as the terminal residue (111). There is evidence for such a modification in *A. nidulans* and in *N. crassa* (109, 110). Although an antibody raised against the detyrosinated form of *A. nidulans* α-tubulin revealed some filaments in immunofluorescence experiments, final conclusions cannot yet be drawn. Attempts to

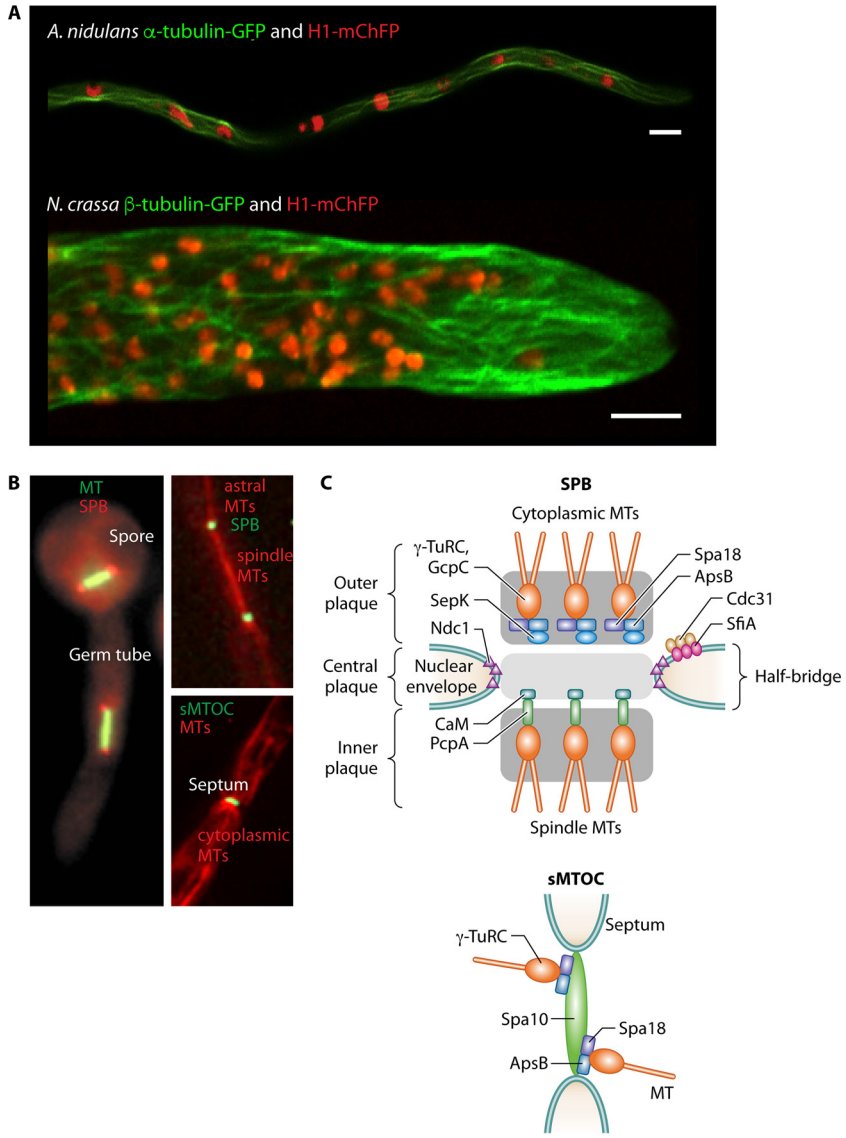

FIG 4 The microtubule cytoskeleton. (A) Microtubule cytoskeleton and nuclear organization in *Neurospora crassa* and *Aspergillus nidulans*. Bars = 5 μm. (Courtesy of Rosa M. Ramírez Cota; reprinted with permission.) (B) Microtubule-organizing centers in *A. nidulans*. Shown is a germling with two mitotic spindles. Microtubules were stained with GFP-tagged alpha tubulin, and the SPBs were labeled with mRFP-tagged ApsB. The right fluorescent pictures show mitotic and astral microtubules emanating from the SPBs (labeled with GFP-Spa10) and cytoplasmic microtubules emanating from a septal MTOC. (C) Schemes of a SPB and a sMTOC.

identify the modification of α-tubulin by mass spectrometry have also failed so far (R. Fischer, unpublished data).

Microtubule-organizing centers. Although the growth of MTs can be observed *in vitro* at high tubulin concentrations without the need for other cellular components, *in vivo* MT polymerization requires large multiprotein complexes, microtubule-organizing centers (MTOCs), for their initiation (112). The *S. cerevisiae* MTOC, the best-studied one in fungi, is embedded in the nuclear envelope and divides at the end of S phase during mitosis (113). The daughter MTOCs are then located at the spindle poles. This structure, called the spindle pole body (SPB), is analogous to centrosomes in higher eukaryotes but does not contain centrioles. Several SPB proteins appear to be conserved in other fungi, although their sequence identity is often quite low. SPBs in *S. cerevisiae* and *S. pombe* consist of at least 18 proteins (114). They are organized in three layers (Fig. 4),

which can be distinguished by electron microscopy. The inner layer polymerizes MTs toward the chromosomes during mitosis, the central layer is required for the embedding of the structure into the nuclear envelope and the duplication of the protein complex prior to mitosis, and the outer layer polymerizes astral MTs during mitosis and interphase MTs in nondividing compartments. Gamma-tubulin, the main MTOC component, was discovered in *A. nidulans* in a genetic screen for suppressors of a beta-tubulin mutation (115–117). Gamma-tubulin associates with other larger core protein complexes, called GCPs (gamma complex proteins) in human cells. In *S. cerevisiae*, only two proteins, Spc97 (GCP2) and Spc98 (GCP3), assemble with γ-tubulin and form the γ-tubulin small complex (γ-TuSC). The γ-tubulin complex in *S. pombe*, *A. nidulans*, and higher eukaryotes more resembles the human protein complex, with GCP4-6 and Mzt1 as additional proteins (118–121). The larger γ-tubulin protein complex is called the γ-tubulin ring complex (γ-TuRC). The γ-tubulin complexes are located in the inner and outer plaques of the SPBs. In addition, there are specific proteins found only at the inner or the outer plaques. Hence, SPBs serve dual functions, MT polymerization during mitosis toward the chromosomes and MT polymerization into the cytoplasm during mitosis (astral MTs) and during interphase. There is good evidence for noncentrosomal MTOCs in fungi (Fig. 4). They were first found in *S. pombe* during cell division and were named equatorial MTOCs (eMTOCs) and interphase MTOCs (iMTOCs) (122, 123). They are transient structures at the cell division site or at the nuclear envelope. Although they contain γ-tubulin and other SPB-associated proteins, the entire protein composition is not yet known. Two additional proteins, Mto1 and Mto2, discovered in *S. pombe* through the analysis of polarity mutants (123, 124), are required for the recruitment of the γ-TuRC to cytoplasmic MTOCs (125, 126). Likewise, several cytoplasmic MTOCs were identified in *U. maydis* (127). The exact composition of non-SPB MTOCs and their activity, regulation, and organization have not yet been completely resolved for any fungus.

In vivo observations of *A. nidulans* revealed that MT plus ends originate from nuclei but also from septa (128) (Fig. 4). γ-Tubulin itself was detectable only at septa in interaction assays with ApsB (orthologue of *S. pombe* Mto1) (129). In a recent study, it was suggested that SPB outer plaque proteins are conserved at septal MTOCs (sMTOCs), in contrast to proteins specific for the central or the inner plaque (130). It was shown that the protein complex is recruited during septation to the constricting ring with the help of two intrinsically disordered proteins, Spa18 (Mto2 in *S. pombe*) and Spa10. These proteins were discovered in *N. crassa* during a systematic screen for septum-associated disordered proteins, some of which were afterwards studied in *A. nidulans* (131, 132). Currently, it is unknown how sMTOCs assembly is linked to the septation machinery. Although sMTOCs resemble eMTOCs of *S. pombe*, a major difference is that eMTOCs are transient structures, whereas *A. nidulans* sMTOCs are persistent structures permanently associated with mature septa. The function of sMTOCs is still being unraveled because the deletion of sMTOC proteins does not cause any severe phenotype with regard to hyphal morphology or growth. There is evidence for noncentrosomal MTOCs at the *N. crassa* hyphal tip. It was shown that a MT plus-end tracking protein (+TIP), an EB1 orthologue, accumulates at the tip and moves in a retrograde manner. Also, bleaching experiments suggested MT polymerization starting from the tip (133, 134). Whether there are MTOCs at septa is still unsolved. Experiments using GFP-tagged β-tubulin revealed MTs on each side of the septum. However, the results were interpreted as MTs moving through the septal pore. Unfortunately, our knowledge of noncentrosomal MTOCs relies on only a very few fungal systems. However, the few examples already suggest major differences. Whereas cytoplasmic MTOCs exist in the basidiomycete *U. maydis*, there is evidence for tip-associated MTOCs in *N. crassa* and for septal MTOCs in *A. nidulans*. Currently, it is impossible to draw a general model for the functions of different noncentrosomal MTOCs. Perhaps the different MTOC repertoire goes along with different MTs and nuclear organizations. In *N. crassa*, compartments are much wider and contain many more MTs and nuclei than those in *A. nidulans* (Fig. 4). In *A. nidulans*, mitoses of nuclei in one compartment are synchronized, and cytoplasmic MTs are largely disassembled during mitoses. One or a very few MTs persist and probably

guarantee cytoplasmic MT-dependent transport during mitosis. Likewise, the growth rate is not reduced during mitosis (110, 135). It remains to be determined if septal MTOCs remain active during mitosis and guarantee MT formation at this stage of the cell cycle. In *N. crassa*, only a few individual nuclei undergo mitosis at a given time, and the cytoplasmic MTs remain intact and support continuous growth. Hence, septal MTOCs would not be required. The different organizations may also be related to the different growth rates of the two fungi. To test these interesting hypotheses, more comparative studies with fungi with different MT organizations and slow- and fast-growing hyphae are required. A better resolution of MT nucleation and MT organization, for instance, with electron tomography, as was established recently for *A. gossypii* (136), is also urgently required.

The microtubule plus ends. Comparably to the large protein complex at the MT minus end, a large number of proteins associated with the growing MT plus end, called +TIPs, control MT plus-end dynamics (137). One such protein is a MT polymerase. Polymerization activity (XMAP215) was shown first in *Xenopus laevis* and then in *S. pombe* (Alp14) (138) and *A. nidulans* (AlpA) (69, 139–142). In addition to the polymerase, a large number of other proteins is associated with the MT growing end. They might function in the polymerization process but are also required for MT plus-end interactions with cortical proteins. Furthermore, some proteins, like the cell end marker TeaA in *A. nidulans* (Tea1 in *S. pombe*), use growing plus ends to travel to the cortex (143). As indicated above, TeaA is part of a cell end marker complex involved in the control of growth direction. More interestingly, TeaA negatively controls AlpA activity once the MT plus end reaches the tip cortex (69). The plus-end interaction with cortical proteins during mitosis has been well characterized and is a process that appears to be conserved in *S. cerevisiae* and *A. nidulans* (144–147). In *S. cerevisiae*, astral MTs emanate from the nuclear envelope into the emerging bud, where they contact myosin V (Myo2) and actin filaments emanating from the bud cortex (148). The myosin motor then pulls the MTs toward the budding neck. Once the MT plus end reaches the cortical membrane, the dynein motor (Dyn1) is activated and pulls the mitotic spindle toward the budding neck (149). In *A. nidulans*, astral MTs pull on each side of the mitotic spindle, causing spindle oscillations. In interphase, MT plus ends of the tip compartment are captured by the actin-myosin system and pulled toward the tip (150). This causes the focusing of the MT array at the tip, which may be important for fast hyphal extension and straight growth (66).

Role of microtubules in intracellular transport processes. Two functions of MTs are already obvious from the above description: the delivery of cell end marker proteins and the movement of large structures, such as nuclei or chromosomes. Other functions depend on MTs as tracks for the motor proteins dynein and kinesin. It has long been suggested that vesicles are associated with motor proteins and are transported along MTs. There are at least two main classes of vesicles, secretory vesicles and early endosomes. Two motor proteins, UncA (Kin3 in *U. maydis*; the motor does not exist in *S. cerevisiae*) and dynein, are the main players in endosome transport, whereas conventional kinesin plays a role in vesicle transportation. The deletion of *kinA* in *A. nidulans* or *kin-1* in *N. crassa* impaired hyphal growth and the delivery of exoenzymes, but it did not abolish hyphal extension (33, 151–153). There is recent convincing evidence that conventional kinesin indeed transports secretion vesicles toward the hyphal tip (85, 154). The deletion of other kinesins, such as UncA, kinesin-7 (KipA), or kinesin-8 (KipB), possibly involved in plus-end-directed transportation, as well as double or triple deletions thereof, did not affect hyphal extension (155). In addition to these observations, which pose doubts about the importance of kinesin and the MT-dependent transport of secretion vesicles, depolymerization experiments revealed that MTs are important during fast hyphal extension but not during germ tube formation and slow hyphal growth (61).

Individual proteins can also be cargoes of kinesin (156). There are two good examples for such transport: the transport of Tea1 along MTs toward the MT plus end by the kinesin-7 Tea2 (KipA in *A. nidulans*) and the transportation of the dynein heavy

chain by conventional kinesin (KinA in *A. nidulans* and Kin1 in *U. maydis*) (156–159). This interplay between different motor proteins complicates the interpretation of phenotypes of the corresponding deletion mutants. Hence, the phenotypes associated with the deletion of conventional kinesin may overlap those associated with the dynein deletion because of the mislocalization of dynein in the kinesin mutant.

Besides the function of endosomes in membrane and membrane protein recycling, novel functions of these organelles were discovered recently. Endosomes serve as multipurpose platforms to transport a variety of cargo, such as lipids, proteins, mRNAs, ribosomes, and even whole organelles (160, 161) (Fig. 5). Characteristic of early endosomes is a distinct lipid composition and marker proteins such as small Rab5-type GTPases (162, 163). To carry out their function, endosomes are transported bidirectionally along microtubules. Kinesin-3-type motors, such as Kin3/UNC-A, mediate plus-end-directed transport (109, 110, 164, 165). Minus-end-directed transport is conducted by cytoplasmic dynein, whose activity is regulated by the dynactin and FHF complex (FTS/Hook/FHIP) (163). Whereas kinesin-3 contains a pleckstrin homology domain for interactions with endosomal lipids (166, 167), components of the FHF complex interact with Rab5 to connect dynein to early endosomes (163, 168).

Initially, it was thought that fungal Rab5-positive early endosomes are mainly involved in the spatial organization of the endocytic process. This was supported by the finding that early endosomes are involved in the membrane recycling of the pheromone receptor Pra1 in *U. maydis* (50). Alternatively, it was postulated that early endosomes might participate in signaling from the hyphal tip toward the nucleus to alter gene expression (169). However, studies of polar growth of infectious hyphae in *U. maydis* revealed novel and unexpected additional functions of endosomal transport (161).

In this phytopathogen, the hyphal growth program is closely linked to infection (170). Infectious hyphae grow with a defined axis of polarity. Cell expansion takes place at the hyphal tip until a defined maximal length is reached (171). This triggers the insertion of basal septa at the opposite pole, resulting in the formation of characteristic empty sections (170) (Fig. 5). Interfering with microtubule-dependent transport results in defects in polar growth: hyphae grow bipolarly, and the insertion of basal septa is delayed (166, 172, 173). The first indication of novel endosomal functions was discovered from an unexpected angle, namely, studying the function of RNA-binding proteins (174, 175). The loss of the RNA-binding protein Rrm4 results in bipolarly growing filaments (158). Interestingly, Rrm4 shuttles along microtubules, suggesting a function in long-distance mRNA transport (158). Studying the motor composition revealed that endosomal motors, namely, kinesin-3 and dynein, mediate Rrm4 transport. Colocalization with endosomal marker proteins confirmed that Rrm4 hitchhikes on early endosomes, and interference with endosomal trafficking disturbs Rrm4 movement (166). In fact, Rrm4 is present exclusively on Rab5a-positive early endosomes, covering almost the entire endosome population. Hence, it serves as an excellent marker protein (176).

Endosomal mRNA transport constitutes a novel mechanism of microtubule-dependent mRNA trafficking (177, 178). Since a link to endoplasmic reticulum (ER) cotransport was already discovered during actin-dependent mRNA transport in *S. cerevisiae* (179), the integration of membrane and mRNA trafficking appeared to be more widespread than initially anticipated (177).

The main function of mRNA transport is the precise determination of protein expression in time and space. In principle, this is achieved by active mRNA transport and the local translation of the encoded protein (178, 180, 181). Among the best-studied examples is the transport of *ASH1* mRNA in *S. cerevisiae*. Here, the RNA-binding proteins She2p and She3p recognize specific localization elements in the cargo mRNA for transport (182). She3p interacts with the myosin Myo4p for active transport toward the growing pole of the daughter cell. Local translation at the tip guarantees that the encoded transcription factor (TF), Ash1p, enters the daughter cell nucleus, resulting in asymmetric gene expression between daughter and mother cells (183). Interestingly, this concept seems to be evolutionarily conserved in fungi. For example, in hyphae

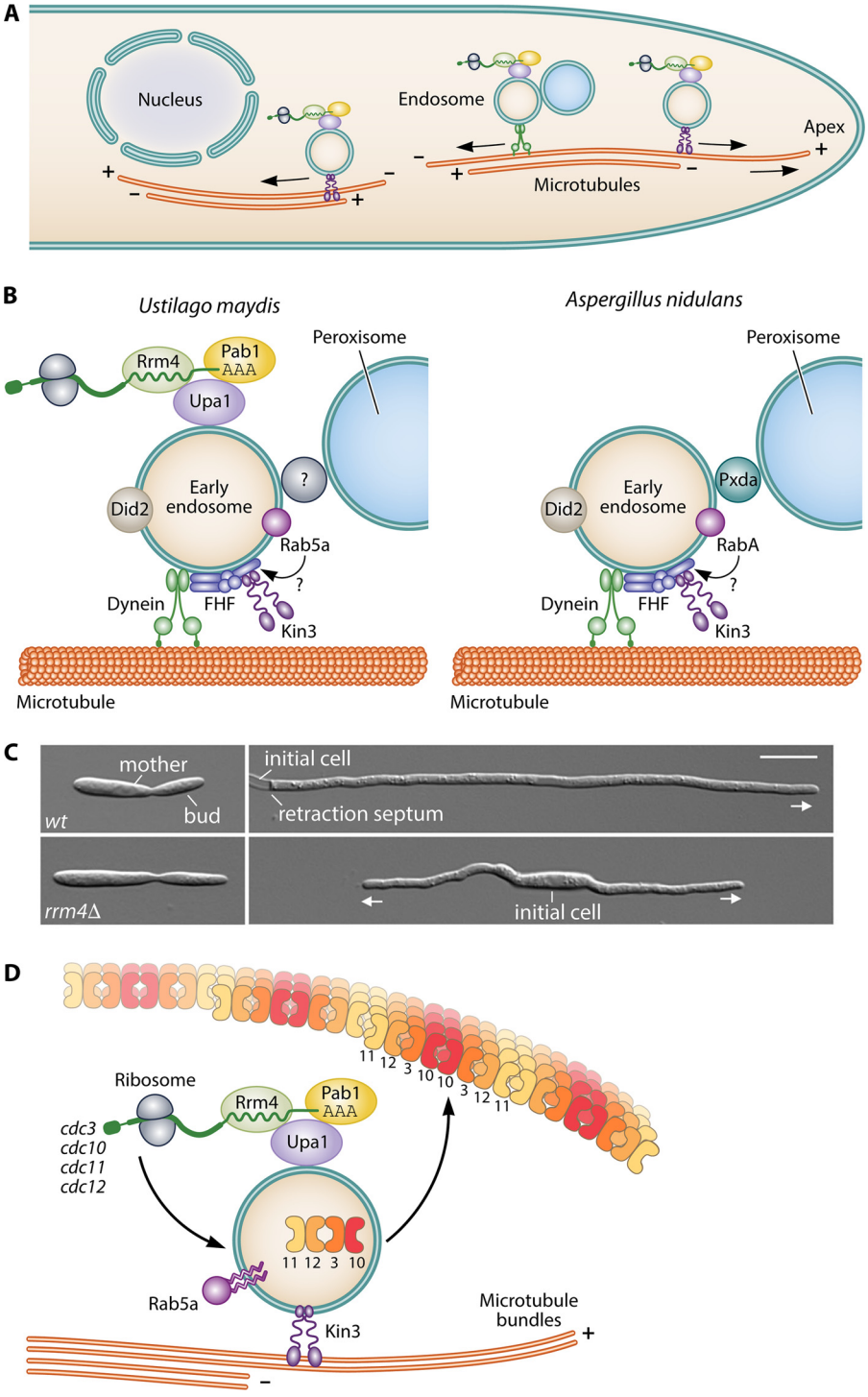

FIG 5 mRNA transport. (A) Scheme of MT-based transport in fungal hyphae. (B) Transport of endosomes in *Ustilago maydis* and *Aspergillus nidulans*. (C) Rrm4 is important for efficient polar growth. (D) RNA transport ensures septin gradient formation. (Panels A and B [left] are modified from reference 445; panel C is modified from reference 193; panel D is adapted from reference 190 with permission.)

from *Candida albicans*, Ash1 accumulates preferentially in the nucleus proximal to the hyphal tip. The loss of She3 causes a mislocalization of *ASH1* mRNA and protein, resulting in defects in hyphal growth (184). A comparable mechanism would explain the predominantly apical accumulation of the transcription factor FlbB in *A. nidulans* (185, 186). Hence, it seems to be a common scheme that fungal transcription factors are

targeted to the first nucleus of polarly growing cells to determine a defined spatial gene expression program.

Studies of Rrm4-mediated mRNA transport revealed that Rrm4 is the main transporter for mRNAs and is needed to distribute mRNA throughout the hyphae, most likely to avoid the formation of artificial protein gradients (187). Consistently, mRNA-associated ribosomes are transported and distributed identically (172, 188). Thus, early endosomes gained a novel function in distributing mRNAs and ribosomes.

Is this mechanism evolutionarily conserved in fungi? So far, it has not been reported that fungi transport mRNAs extensively. Alternatively, other fungi, such as *A. nidulans* and *N. crassa*, could rely on cytoplasmic streaming or nuclear movement to distribute their mRNAs. In the latter process, the whole organelle of mRNA synthesis, instead of the product, is transported. Interestingly, in *Drosophila melanogaster*, intensive ooplasmic streaming is needed during development for the distribution of cellular material throughout the whole oocyte (178).

The question of whether the endosomal mRNA transport machinery also transported specific mRNAs was addressed by *in vivo* UV cross-linking experiments using Rrm4 as bait. Distinct mRNAs were identified, encoding the example chitinase *cts1* or the septin *cdc3* (187, 189). Interestingly, Cts1 export is disturbed in the absence of Rrm4, suggesting a molecular link between its secretion and endosomal mRNA transport (189).

Studies of *cdc3*Δ mutants revealed that hyphae initially grow out bipolarly. By using RNA live imaging, it was shown that *cdc3* mRNA is transported on endosomes. The encoded protein, Cdc3, also accumulates on Rrm4-positive early endosomes and forms higher-order filaments with a gradient emanating from the hyphal tip. Importantly, both subcellular localizations of Cdc3 depend on Rrm4 (188). Since the presence of both *cdc3* mRNA and ribosomes at early endosomes is dependent on Rrm4, the local translation of *cdc3* mRNA most likely loads the cytoplasmic surface of endosomes with Cdc3 protein for transport toward the hyphal tip (161, 188). Consistently, all four septin mRNAs and proteins localize to early endosomes and are assembled in higher-order filaments. Thus, the surface of endosomes is used for the assembly of heteromeric septin complexes, and these complexes are transported for the formation of higher-order structures (190). In accordance, the membrane-assisted assembly of heteromeric septin structures was recently shown, and septins were able to recognize specific membrane curvatures (191, 192). Importantly, this is yet another new function of early endosomes: the transport of newly synthesized proteins and the assembly of heteromeric protein complexes.

But how are mRNPs attached to endosomes? A novel linker protein called Upa1 that interacts with Rrm4 and the poly(A)-binding protein Pab1 was discovered. This protein contains a FYVE domain for endosomal contact and thus recruits Rrm4 and associated mRNAs to endosomes (Fig. 5). A loss of function causes no defects in general endosomal functions but causes defects in the recruitment of Rrm4, septin mRNA, and septin protein. As expected, this results in defective septin filament formation and in disturbed hyphal growth (193).

Recently, it was also discovered that early endosomes transport whole organelles, such as peroxisomes (160, 194). In *A. nidulans*, an adaptor protein, PxdA, that connects peroxisomal cargo to endosomes was found. Interestingly, homologues are present in other fungi, such as *Podospora anserina* and *N. crassa*, suggesting an evolutionary conservation of the mechanism. Thus, it appears to be common that unconventional endosomal cargoes, such as mRNPs and peroxisomes, are attached to endosomes by specific adaptor proteins to guarantee transport (160) (Fig. 5).

How are the classical functions of early endosomes in endocytosis and their new functions in long-distance transport coordinated? The ESCRT (endosomal sorting complex required for transport) regulator Did2 plays an important role (167). In the absence of Did2, the maturation of early endosomes is disturbed, resulting in an altered lipid composition. This causes the altered attachment of molecular motors as well as mRNA cargo. The resulting hyphae exhibit defects in Rrm4-dependent mRNA transport and

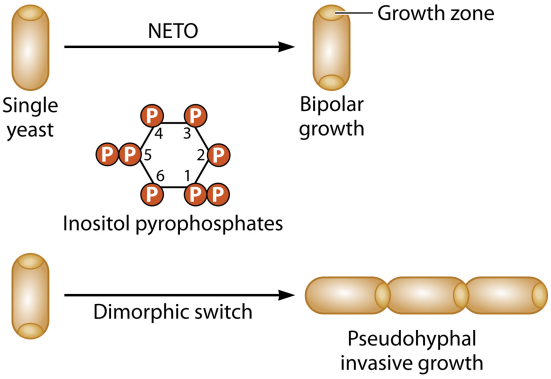

FIG 6 Inositol pyrophosphate-regulated cell morphogenesis in the fission yeast *Schizosaccharomyces pombe*. The transition from mono- to bipolar growth (NETO [new end take off]) requires inositol pyrophosphate generated by the *S. pombe* Asp1 protein, as does the switch from single-celled yeast growth to invasive pseudohyphal growth (dimorphic switch).

septin filament formation and grow bipolarly (167). Thus, the ESCRT regulator appears to orchestrate endocytosis and long-distance transport of endosomes.

In summary, research on different fungal model systems has greatly advanced the characterization of the functions of early endosomes as universal carrier systems (195). New cargoes, such as mRNAs, ribosomes, septins, and peroxisomes, as well as novel concepts, such as endosome-coupled translation or endosomal septin assembly, were uncovered. Moreover, new mechanisms of cargo loading mediated by specific linkers were described. This underpins the strength of comparing fungal model systems to reveal novel and unifying concepts in cell biology.

Inositol pyrophosphates regulate microtubule dynamics and morphogenesis. Recently, a class of high-energy molecules, namely, inositol pyrophosphates (IPPs), was identified to play an important role in MT dynamics (196). IPPs affect numerous cellular processes in eukaryotes by either (i) protein pyrophosphorylation or (ii) reversible binding to a protein-protein complex (197, 198). Two highly conserved classes of enzymes can generate IPPs: the IP6K family and the Vip1 (PPIP5K) family. Vip1 family members are bifunctional enzymes consisting of an N-terminal kinase domain and a C-terminal pyrophosphatase domain, which *in vitro* specifically hydrolyze the IPPs generated by the Vip1 kinase domain (196, 199).

It was first shown in *S. pombe* that the kinase function of Vip1 family members (in *S. pombe*, the Vip1 member is named Asp1) was required for correct polarized growth. This yeast cylindrical cell shape is maintained by restricting growth zones to the cell ends. The absence of Vip1-generated IPPs resulted in an inability to activate the second growth zone in growing cells and abolished correct growth zone definition after reentry into the cell cycle (196). Furthermore, the morphological transition from the yeast cell form to the pseudohyphal invasive growth form, which represents a foraging response, depends on Vip1-made IPPs (200) (Fig. 6). The absence of the gene encoding the Vip1 orthologue in *A. nidulans* (VlpA) resulted in the mispositioning of the second germ tube, demonstrating that IPPs have a role in the selection of the correct growth zone in *A. nidulans* (196). Interestingly, in the distantly related fungus *U. maydis*, the absence of the Vip1 kinase UmAsp1 resulted in abnormally shorter hyphal filaments that were often bipolar. In addition, the morphology of cells proliferating in the yeast-like growth form was also aberrant (196). Thus, IPPs generated by Vip1 family members play a role in the morphogenesis of filamentous fungi.

How, then, do IPPs modulate fungal morphogenesis? Analyses of *S. pombe* revealed that Vip1 proteins control MT dynamics (196, 201). In this yeast, interphase MT arrays are being polymerized from iMTOCs in the vicinity of the nucleus and grow along the long axis of the cell to the end. Upon reaching the cell end, MT dynamics changes from polymerization to pausing, followed by depolymerization. Alteration of intracellular IPP

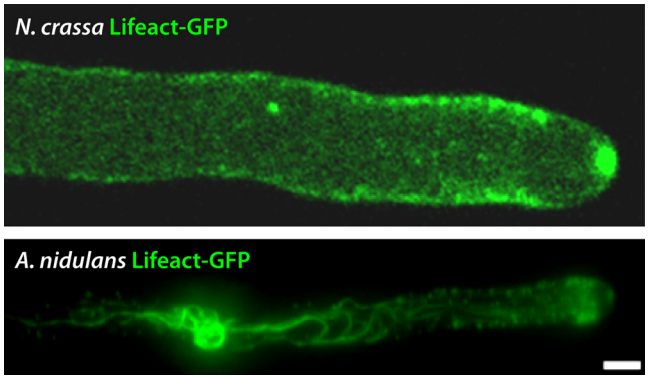

FIG 7 Actin organization at hyphal apical and subapical regions in *N. crassa* and *A. nidulans*. Bar = 5 μm. (Courtesy of L. Quintanilla and B. D. Shaw; reprinted with permission.)

levels resulted in changes of the residence time of the MT plus end at the *S. pombe* cell end: cells that were unable to generate Vip1-made IPPs showed a significantly shorter pausing time at the cell end than that of wild-type cells, while cells with higher-than-wild-type IPP levels showed the opposite effect (196). Similar observations were made for *U. maydis*. The deletion of the gene encoding UmAsp1 resulted in aberrant yeast-like cells with interphase MT plus ends that exhibited a significantly reduced residence time at the cell end (196). Furthermore, fewer MT plus ends reached the hyphal tip in *A. nidulans vlpA* deletion strains than in wild-type strains, indicating altered MT dynamics. Thus, modulation of MT dynamics, in particular the cross talk between MT plus ends and growth zone regulation, appears to be a conserved task of fungal Vip1 proteins.

The Actin Cytoskeleton

Actin, one of the most abundant proteins in eukaryotes, plays a major role in diverse cellular functions. In filamentous fungi, F-actin and its associated proteins are involved in growth, the spatial regulation of organelles, exocytosis, endocytosis, and cytokinesis (55, 63, 89, 202, 203). A disruption of F-actin function revealed that a well-polymerized actin cytoskeleton is essential for apical growth, hyphal shape, and polarized secretion in different fungi (47, 204–207). Actin-binding proteins (ABPs), such as fimbrin, coronin, the Arp2/3 complex, and myosin II, regulate F-actin arrangement and organization. The different higher-order F-actin structures found in fungi, patches, cables, and contractile rings, are responsible for distinct processes (47, 57, 208).

Actin at the hyphal tip. The population of F-actin in the Spitzenkörper has been suggested to regulate secretory vesicle delivery to the plasma membrane in the apical dome (9, 89, 202, 209). By using the actin reporter Lifeact (210), it was possible to confirm previous reports of actin accumulation at hyphal tips (57, 211–213) (Fig. 7). Although it was not possible to observe single F-actin cables in the Spitzenkörper by wide-field fluorescence microscopy, F-actin appeared as a dense meshwork of cables embedded in other components of the Spitzenkörper by TEM (214). Presumably, contractile forces within the Spitzenkörper are needed not only to maintain its organization but also to account for some minor movements during growth. MYO-2, a class II myosin, has also been found as part of the core of the Spitzenkörper. Nevertheless, its function as a contractile force needs to be explored. Clearly, F-actin is essential for a well-organized tip growth apparatus, a conclusion supported by the disassembly of the polarisome and the Spitzenkörper following treatment with latrunculin A or cytochalasin D (95). Tropomyosin is another component of the actin cytoskeleton in the hyphal tip. This protein stabilizes F-actin and controls F-actin mechanics, regulating the interaction between F-actin and myosins (215–217).

The subapex and the endocytic region. Patches of actin accompanied by several proteins (e.g., fimbrin, the Arp2/3 complex, and coronin) mediate endocytosis and are found along hyphae of different fungal species but concentrate mainly at the subapical

endocytic collar (57, 211–213) (Fig. 7). This subapical arrangement seems to be conserved in different species of filamentous fungi, such as *N. crassa* (57, 211, 218), *A. nidulans* (213), *Sclerotium rolfsii* (219), *Allomyces macrogynus* (220), *Mucor rouxii* (209), and *Colletotrichum graminicola* (212). The endocytic patches serve to recycle plasma membrane and polarity markers (55, 56, 221, 222). Actin patches are assembled at the plasma membrane as part of the last step for coating of the endocytic vesicles (223). In *N. crassa*, abundant actin patches were localized throughout the cell cortex. Total internal reflection fluorescence microscopy (TIRFM) demonstrated an abundance of cortical patches in the subapex and in basal regions of hyphae, which are difficult to determine by confocal microscopy (57). The actin coat drives the invagination of endocytic vesicles to overcome the internal turgor of fungal cells, and it remains present after their scission (224). This coat is gradually lost as the vesicles are transported to endosomal compartments (225).

The composition of patches in filamentous fungi is consistent with what has been reported for yeast cells (89, 226–232). In *S. cerevisiae*, fimbrin (Sac6)-null mutants have severe defects in the endocytic uptake of α-factor. Also, in *A. nidulans* and *N. crassa*, fimbrin mutants are unable to produce colonies and cannot internalize the endocytic marker FM4-64 (55), demonstrating that fimbrin is essential for endocytosis (231, 232). The Arp2/3 complex, an essential component in most fungi, is found exclusively in actin patches, where it nucleates F-actin branches (233).

Endocytic actin patches have patterns of slow or fast movement, in either an anterograde or retrograde direction, in yeast (225, 234, 235) and in filamentous fungi (55, 57). In cells treated with latrunculin A and cytochalasin D, patch movement stopped, suggesting that patch movement in the cortex occurs via F-actin cables (57, 225, 234). Endocytic actin patches of *N. crassa* display the highest speeds reported for fungal cells (3.35 μm/s), compared to those for *A. nidulans* (0.19 to 0.56 μm/s) (55), *S. cerevisiae* (0.31 μm/s), and *S. pombe* (0.3 μm/s) (235, 236). This value is an order of magnitude higher and is probably related to the high growth rate of *N. crassa*.

In addition to patches, F-actin cables are found along hyphae, probably serving as tracks for the transport of various cargoes, including secretory and endocytic vesicles, mRNAs, and Golgi equivalents, to the sites of growth and cell division, as shown in cells of *S. cerevisiae* and, more recently, in hyphae of *Candida albicans* (92, 184, 208, 237, 238). In some fungal species, these cables are arranged in the cytoplasm close to the cell cortex (57, 239).

The septal actomyosin tangle and contractile actomyosin ring. Septum formation is another F-actin-dependent process. The mycelia of higher fungi are made of multinucleated hyphae with regularly spaced septa. A study of a wild strain of *N. crassa* revealed that a new septum arises at a defined position $\sim$165 μm from the growing tip of mature hyphae, indicating that a size-sensing mechanism probably determines the regularity of septation (240–243). The number and positioning of nuclei as well as cell cycle signaling regulate septum placement in *A. nidulans*, even though not every nuclear division generates a septum (244, 245). Septum formation involves the regulation of mitotic signaling, cytokinesis, and cell wall biosynthesis (242, 246, 247). In different fungi, there is a conserved temporal and spatial pathway to assemble F-actin-containing structures essential for septation (243, 248–252). The first discernible structure in septation in *N. crassa* is the septal actomyosin tangle (SAT), an elaborate tangle of F-actin cables associated with tropomyosin and a class II myosin (MYO-2). The SAT establishes the septation site several minutes before actual septum formation begins (243). The SAT is formed very close to the cell cortex as a collection of extended helicoidal cables that are compressed longitudinally toward a midpoint, forming a proto-contractile actomyosin ring (CAR), a ringlike structure that is not in its final place. The proto-CAR progresses into the fully mature CAR, which drives the final step of a conserved process called cytokinesis. This process follows the same general principle in all organisms; however, septation in hyphae is usually an incomplete event allowing communication between compartments through a central pore in the septum (247, 253). The transformation of a SAT into a CAR in *N. crassa* brings to mind the dynamics

of actin during septation in *S. pombe* (254, 255). The corresponding process of SAT formation and conversion to CAR in yeasts is called the "search, capture, pull, and release" model (254). Although there are some differences, in *S. pombe*, there is an accumulation of the anillin-like Mid1 nodes in the division site almost 1 h before mitosis (255–257). When mitosis begins, Mid1 recruits the class II myosin Myo2, forming a wide band of nodes together with Rlc1, the formin Cdc12, and the tropomyosin Cdc8 (254, 258–261). In *N. crassa*, there is no Mid1 homologue, and no other known protein seems to form nodes close to the septation site. However, MYO-2 appears before there is any sign of plasma membrane ingrowth, and its appearance coincides with that of actin. Another discrepancy is that the so-called landmark proteins BUD-4, BUD-3, RHO-4, and formin BNI-1 are not present during SAT formation and transformation into the CAR in *N. crassa* (243). The arrival of BUD-4 and the formin BNI-1 coincides with the maturation of the proto-CAR into a CAR, suggesting that these two proteins have a critical role in this process (243).

The role of actin patches in septation is less clear. Conceivably, their role is to promote endocytic uptake required to remove a presumed excess of membrane produced by the secretion process that takes place at the septation sites in support of cell wall construction and membrane expansion.

MOLECULAR MECHANISMS UNDERLYING HYPHAL DIFFERENTIATION

The apical extension and polarized growth of filamentous fungi described above may continue indefinitely as long as nutrients and space are available. Under stressful conditions, such as nutrient limitation, fungi form asexual or sexual reproductive structures that produce spores. The forms of these spores vary, but the outcome is similar: dehydrated, metabolically inactive cells that contain diverse accumulated metabolites and are insulated from the environment by a thick cell wall and coats. The process of spore formation is complex, normally takes several hours to a few days, and is usually asynchronous, and the spores generally require a maturation period before they can germinate.

Transitions from Vegetative Growth to Asexual Development

Because asexual development (conidiation) has been intensively studied in *A. nidulans* and *N. crassa*, we focus on these model fungi, but we also refer to work done with other fungal species. *A. nidulans* conidiation was reviewed previously (262–264). Here, we concentrate on the polarity changes that occur during this process and the cell signaling events that initiate it. Conidiation involves the development of conidiophores, which in a colony occurs after a relatively short period of growth and at an air interface. Thus, colony growth occurs at the edges, while conidiophores are being produced slightly behind the colony border. This strategy allows both substrate colonization and reproduction before the fungus runs out of nutrients. The *A. nidulans* conidiophore develops as a polarized outgrowth of a hyphal compartment delimited by two septa, called the foot cell. This structure develops into a thickened wall hypha that grows perpendicularly to the medium, toward the air phase. After a fixed length is reached, a dramatic shift from polar to isotropic growth occurs, which results in the formation of a globular multinucleated structure, from which uninucleated buds called metulae are formed. By other budding processes, each metula develops two uninucleated conidiogenic cells called phialides. Each phialide produces about 100 uninucleated conidia through periodic budding cycles (Fig. 8). In the presence of nutrients, conidia germinate and go back to polarized growth by producing a germ tube in a process that is tightly linked to nuclear division. Similar developmental patterns, which clearly involve major changes in cytoskeleton and gene expression patterns, occur in other Aspergilli and phialoconidiogenic fungi such as human- and plant-pathogenic fungi, including *Talaromyces* (formerly *Penicillium*) *marneffei* (265) and *Fusarium graminearum* (teleomorph: *Gibberella zeae*) (266).

There is a clear asymmetry between the phialides that contain a nucleus capable of undergoing repeated mitotic divisions and the newly developed conidia that inherit a

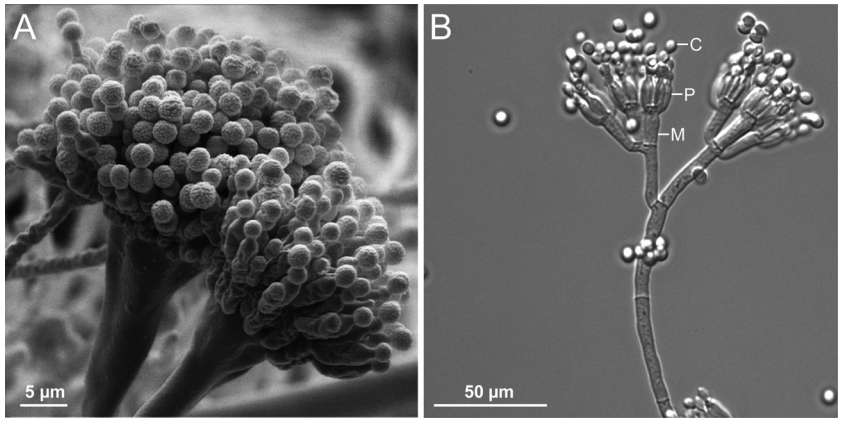

FIG 8 The activity of the transcription factor BrlA results in different conidiophore morphological patterns in *Aspergillus nidulans* (scanning electron micrograph) (image by G. H. Braus) (A) and *Penicillium chrysogenum* (light microscopic image) (image by U. Kück) (B). The *P. chrysogenum* conidiophore shows a simpler structure, lacking a multinucleated vesicle and producing fewer metulae (M), phialides (P), and conidia (C).

nucleus that becomes arrested in the G_1 phase of the cell cycle. The sharp segregation of catalase A activity between conidiating tissue and conidia (267) is a notable example of this functional and spatial asymmetry as related to reactive oxygen species (ROS) metabolism. Little is known about the mechanisms that determine such different cell fates. However, it is known that the pathway composed of the mitogen-activated protein kinase (MAPK) SakA/HogA (268, 269), the transcription factor AtfA (270), and the calmodulin-dependent kinase (CAMK) SrkA (271), which in hyphae regulate oxidative and other types of stress responses, play critical roles in cell cycle arrest, conidium development, and germination. The constitutive phosphorylation of SakA, for example, as induced by the fungicide fludioxonil, results in lethal cell cycle arrest (270, 272, 273). SakA is activated during conidiation by unknown mechanisms that might involve oxidative and/or osmotic stress and/or phosphatase inactivation. Bimolecular fluorescence complementation (BiFC) experiments showed that active SakA and AtfA do not present nuclear interactions at the vesicle stage, and this is consistent with the fact that phosphorylated SakA would result in cell cycle arrest, preventing nuclear division. However, SakA and AtfA show nuclear interactions in metulae, phialides, and conidia (270). More importantly, SakA dephosphorylation was shown to be necessary for polar growth establishment and nuclear division during the germination of conidia (270). On the other hand, the central cell cycle regulator NimX (Cdc28 in *S. cerevisiae*), the cyclin NimE, and the Pcl-type cyclin PclA are transcriptionally upregulated during conidium formation (274, 275). This suggests an adaptation of the cell cycle to the specific developmental requirements during conidium formation. The exact timely and spatial control of the expression of conidiophore-specific genes and the adjustments in the expression of other genes require the action of a network of transcriptional factors.

It has long been known that the Zn finger transcription factor BrlA is essential and constantly required for the whole conidiation process but is dispensable for sexual development (262–264). Indeed, in *brlA*-null mutants, conidiophore stalk polar growth continues indeterminately, and no isotropic growth is produced. Moreover, conidiophore cell shape can be drastically altered by mutations that modulate the *brlA* expression pattern (276), as in *stuA*-, *medA*-, and *abaA*-null mutants, or by high *brlA* levels induced by forced expression or by carbon source starvation (277). High *brlA* expression levels result in the bypass of the vesicle and metula stages and the direct production of phialides that form one or a few conidia, while the forced expression of *brlA* during conidium germination results in a failure to establish polar growth. Given the central role of *brlA* in polar growth and conidiation, research devoted to this subject has addressed the questions of how BrlA regulates polar growth and

what controls *brlA* expression. Studies of homologues of the yeast bud site selection proteins Bud4 and Axl2 in *A. nidulans* showed that Bud4 is involved in septum formation in both hyphae and conidiophores (278). In contrast, Axl2 has no obvious function in hyphal growth; it is localized at the phialide-spore junction, where it appears to promote septin recruitment. More importantly, Axl2 is required for phialide morphogenesis, and BrlA and AbaA regulate its expression. The inactivation of Axl2 in *F. graminearum* resulted in decreased conidiation, and in both *A. nidulans* and *F. graminearum*, it produced a clear derepression of sexual development.

One approach aimed at understanding what controls *brlA* expression consisted of the isolation of *fluffy* mutants that macroscopically show a proliferation of aerial hyphae and decreased conidiation (279–281). This led to the identification and characterization of the *brlA* upstream activators FluG, FlbA, FlbB, FlbC, FlbD, FlbE, TmpA, AfeA, and TmpB (262–264). More relevant to this review are FlbB, FlbE, FluG, TmpA, AfeA, and TmpB, which play unique roles in hyphal polarity and in the signaling events that initiate conidiation. FlbB, a bZIP protein, is the first transcription factor known to localize at the tip of hyphae and growing metulae (186). Such a localization seems necessary before FlbB can be localized in nuclei, which, together with FlbD, is required to induce *brlA* expression (282). Notably, FlbB shows higher concentrations in the most apical nuclei and decreasing concentrations in successive nuclei (186). The actin cytoskeleton and the small protein FlbE are required for FlbB transport and accumulation at the hyphal tip (283).

Instead of being regulatory proteins, FluG, TmpA, AfeA, and TmpB are enzymes involved in the production of extracellular chemical signals that regulate *brlA* expression and conidiation. FluG is a glutamine synthetase I-like-domain-containing protein. *fluG* overexpression induces *brlA* activation and conidiation in liquid-submerged cultures, while *fluG*-null mutants fail to produce an extracellular signal (284, 285) identified as a diorcinol-dehydroaustinol adduct that rescues *fluG* mutant conidiation defects (286). TmpB contains adenylation and oxidoreductase domains, an architecture shared by the bimodular ChNPS12/ETP nonribosomal peptide synthetases (NRPSs), and its inactivation produces a *fluffy* phenotype in the center of the colony but not in the periphery (280). This and the fact that dehydroaustinol is produced through a polyketide synthase (PKS)-mediated pathway (287) show that both PKSs and NRPSs are involved in the production of different sporulation signals. This is consistent with the fact that the partial inactivation of the phosphopantetheinyl transferase CfwA/NpgA, required for the activation of all PKSs and NRPSs, results in an almost aconidial phenotype (288). The inactivation of the *gmcA* gene, encoding a putative glucose-methanol-choline oxidoreductase, also results in a clearly *fluffy* phenotype but only at alkaline pH. Colony cross-feeding experiments showed that GmcA is necessary for the synthesis of a metabolite that can be transmitted extracellularly (289), different from the sporulation signals produced through the FluG and the AfeA-TmpA pathways (279, 280). The regulation of conidiation by secondary-metabolism-derived signals is conserved in other fungi, such as *Fusarium fujikuroi* (290). Nevertheless, the mechanisms by which all these signals regulate conidiation are unknown.

A different type of molecule, the oxylipins called psi factors, was long ago shown to regulate the balance between asexual and sexual sporulation in *A. nidulans* (291, 292). Since then, a large body of research has been conducted in the fungal oxylipin field (293, 294). Oxylipins are oxidized fatty acids with ubiquitous, well-documented effects on plant (i.e., jasmonic acid), animal (eicosanoids), and fungal (psi factors) signaling. In fungi, some oxylipins are produced by lipoxygenases, cyclooxigenases, and monooxygenases, while others are produced nonenzymatically or by as-yet-unidentified pathways. Perhaps because of this, there is a close link between ROS (295–298), Ca^{2+}, and oxylipin signaling during development (282, 294, 299) and regeneration and wounding responses (300–302). Indeed, the role of oxylipin signaling in cross-kingdom communication, particularly between fungi and plants, has become of great interest (294), although the role of ROS in such signaling is still little studied.

The enzymes AfeA and TmpA are part of a single pathway that might be involved in oxylipin biosynthesis and is required for *brlA* expression and conidiation (280). TmpA is a plasma membrane oxidoreductase (279), while AfeA is an adenylate-forming enzyme related to fatty acyl-CoA synthetases and the plant 4-coumarate ligase-like enzyme At4g05160, involved in fatty acid activation and jasmonic acid biosynthesis. AfeA is localized in the plasma membrane, and like the oxylipin biosynthesis enzyme fatty acid dioxygenase PpoA, it is also localized in lipid bodies (280). The role of AfeA in oxylipin metabolism is further supported by the fact that *afeA*-null mutants show a clearly altered oxylipin profile (M. Reverberi and J. Aguirre, unpublished data). As in mammalian and plant cells, fungal oxylipins exert at least some of their functions through G-protein-coupled receptors that regulate intracellular cAMP levels and are somehow related to the expression of *brlA* and other developmental genes (303). As critical as the *brlA* gene is for *A. nidulans* conidiation, its function is conserved mainly among *Aspergillus* and *Penicillium* species, and it is notably absent in nonphialidogenic fungi such as *N. crassa*. In contrast, the *medA*, *abaA*, *wetA*, and *stuA* genes and their role in conidiation functions are conserved in many fungal species (264, 304).

In contrast to *Aspergillus* and *Penicillium* phialoconidiation, macroconidiation in *N. crassa* involves the transition from growth by hyphal tip elongation to growth by repeated apical budding, resulting in the formation of chains of proconidia that are later separated by cross-walls (blastoarthrospores). The entire conidiation process in *N. crassa* goes through different stages, each of which is triggered by a hyperoxidant state (HO), an unstable, transient state in which ROS are formed in amounts that surpass the antioxidant capacity of the cell (295, 297, 305). Within 3 min after air exposure, *N. crassa* hyphae in direct contact with air develop a HO, and as a response, hyphae adhere to each other in the course of 40 min. In liquid culture, the adhesion of hyphae occurs during pre-stationary-phase growth, before the carbon source is depleted; hyphae rapidly use glucose and secrete a saccharide, and its polymerization requires the presence of iron (305). Similar to bacteria, fungi secrete polysaccharides, proteins, and lipids and form an extracellular matrix (biofilm) under stress conditions. A nuclear magnetic resonance (NMR) analysis of the *A. fumigatus* extracellular matrix indicated that modified polysaccharides are the main component (306), as found in *Vibrio cholerae* by those same authors. Although dioxygen diffusion is limited in bacterial (307) and fungal (308) biofilms, how these changes trigger differentiation at the cellular level is largely unknown. However, it has been shown that the microtubule cytoskeleton undergoes major changes in *A. nidulans* biofilms (309).

N. crassa air-exposed, adhered hyphae are only temporarily protected: they undergo a HO again and become highly vacuolated. Dying cells serve as a substrate for aerial hyphal growth (310) in a process where hydrophobins are instrumental. The transcription factor FL (*Fluffy*) transcribes the *eas* (easily wettable) gene, encoding the hydrophobin of aerial hyphae and conidia (311). The self-assembly of the hydrophobin EAS into fibrillar rodlets occurs spontaneously at hydrophobic-hydrophilic interfaces, forming an amphipathic monolayer (312). Similarly, in *Streptomyces coelicolor*, after stress and the death of the substrate mycelium, "aerial hyphae" that express the hydrophobin-like proteins chaplin and rodlin are formed; both cell death and morphogenesis depend on oxidative stress (313). As O_2 normally diffuses from the extracellular aqueous medium into the cytoplasm, hydrophobins might hinder direct O_2 diffusion from air, forcing O_2 ministration to the aerial hyphae mainly through the mycelial substrate. Cholesterol in mammalian cells (314, 315) and hopanoids in bacteria (316) also reduce plasma membrane O_2 permeability. The transcription repressor CSP-1 (conidial separation 1) regulates ergosterol synthesis and membrane lipid composition in *N. crassa* (317). Oxidative stress releases CSP-1 repression, allowing ergosterol gene expression. Various azoles, such as miconazole and fluconazole, besides inhibiting lanosterol 14-alpha-demethylase (*erg11*) of the ergosterol biosynthesis pathway, generate oxidative stress in fungi (318, 319) and derepress CSP-1 in *N. crassa* (320). Furthermore, ergosterol is essential for spore formation in *Fusarium graminearum* (321). Similarly, hopanoids (bacterial steroids) are synthetized during the transition of

the substrate to aerial hyphae in *Streptomyces coelicolor* (322). Thus, such modifications of the cell wall and the plasma membrane could reduce O_2 diffusion into cells.

Sustained by the substrate-adhered hyphae, *N. crassa* aerial hyphae grow apically and ramify, but another HO then develops after 8 h of air exposure, leading to conidium formation (305). After three events of oxidative stress, in which NAD(P)H and glutathione (GSH) become increasingly oxidized, these compounds are found oxidized in *N. crassa* conidia (323). Germination breaks cell insulation: O_2 enters conidia and generates a HO, which is controlled by nutrient utilization (324). Most conidial mRNA is degraded and resynthesized before polarized growth restarts (325).

Growth and conidiation can alternate with a time period of 20 to 22 h in *N. crassa* WT strains under stress or in some mutant strains, such as the *ras-1*^{bd} strain and a *sod-1* strain (326, 327). ROS greatly influences cyclic conidiation: menadione, a superoxide-producing compound, reduces this time period in WT and *sod-1*Δ strains, and the overexpression of *sod-1* results in delayed conidiation (328, 329). Menadione also reduces this time period in the *ras-1*^{bd} strain with increasing light intensity (330). H_2O_2 (0.5 to 1 mM), besides light, activates the white collar complex; transcribes the *cat-1* gene, the main catalase of conidiation (331); and generates a *cat-1* mRNA cycle (332). Consistent with the role of ROS in cyclic conidiation, many light-induced genes in *N. crassa* encode antioxidants (333).

Fruiting Body Formation

The formation of fruiting bodies during the sexual reproductive cycle has been studied in zygomycetes, ascomycota, and basidiomycota. Fruiting body sizes range from several micrometers to the macroscopic scale of mushrooms. Morphogenesis requires the differentiation of mycelia into specialized tissues that protect the products of meiosis, the meiospores, and optimize their release in the environment. The regulation of morphological and physiological changes associated with fruiting body formation is under polygenic control and is best understood for genetically accessible ascomycetes. Here, we mainly describe work with the homothallic (self-compatible) ascomycetes *Sordaria macrospora* (genome-wide expression patterns) and *A. nidulans* (posttranslational modifications) but also refer to work done with other filamentous ascomycetes.

Within the Ascomycota, most members of the Pezizomycotina form a spore-bearing hymenium within complex fruiting bodies (ascocarps). However, even among the Taphrinomycotina, which harbor mostly unicellular yeasts, species like *Neolecta irregularis* form ascocarps. Genome sequencing showed that *Neolecta irregularis* and Pezizomycotina share about 1,050 genes, not present in ascomycetous yeasts, with enriched functions related to complex multicellularity (334). Generally, five types of ascocarps are distinguished within the Pezizomycotina (apothecium, cleistothecium, gymnothecium, perithecium, or pseudothecium). For example, flask-shaped perithecia occur in *N. crassa* and *S. macrospora*, and closed cleistothecia occur in *A. nidulans* and diverse *Aspergillus* and *Penicillium* species (335). Fruiting body formation requires a hierarchy of gene network expression patterns coordinated in time and space and is dependent on major posttranslational modifications. The sexual cycle and initiation of fruiting body formation are well studied in *N. crassa*. In this heterothallic fungus, "A" and "a" mating-type (MAT) genes are distinguished, each of which is able to generate male (macro- or microconidia) or female (protoperithecium) gametangia. The protoperithecium generates an uptake hypha, the trichogyne, which can fuse with a male gamete. Self-fertilization is prevented by an incompatibility system, which guarantees that only two cells derived from strains with the opposite mating type will fuse. This is a prerequisite for the generation a heterokaryon, with genetically different nuclei. After fertilization, protoperithecia develop into perithecia by enlarging and developing at least 15 different tissues that constitute the fruiting body. Within perithecia, a generative tissue (hymenium) is generated, where the two genetically different nuclei migrate into hook-shaped crozier cells. After coordinated mitosis, three cells are generated in each crozier, an upper binucleated cell and two flanking cells, containing only a single

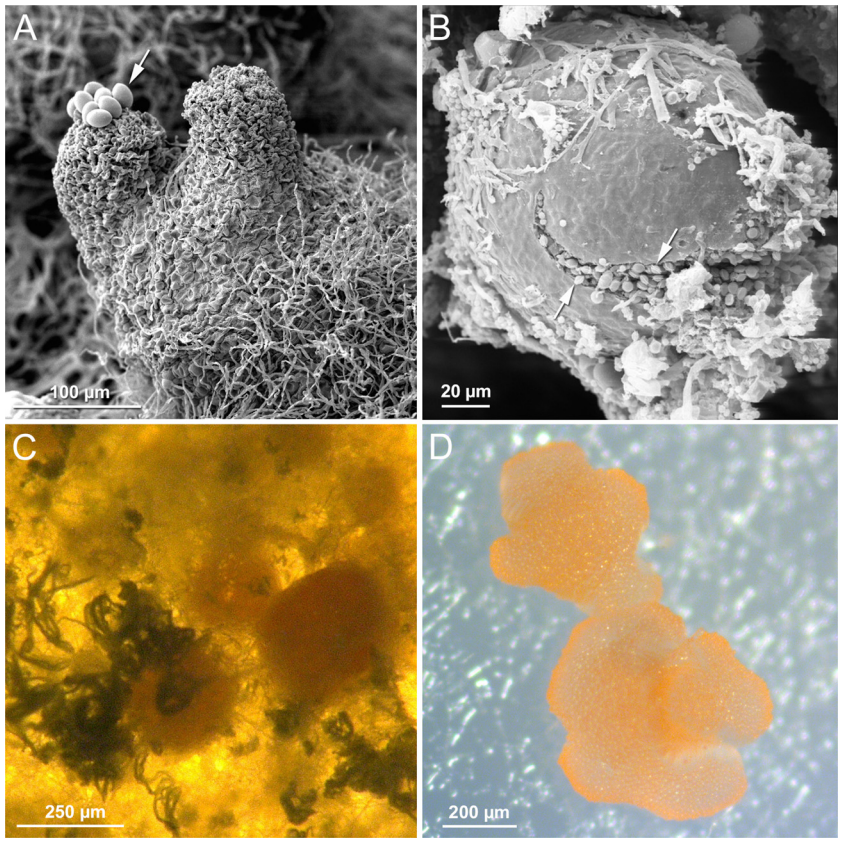

FIG 9 Fruiting bodies from diverse ascomycetes. (A) Perithecia from *Sordaria macrospora*. (Image by U. Kück.) (B) Cleistothecium from *Aspergillus nidulans*. (Image by G. H. Braus.) (C) Cleistothecia from *Penicillium chrysogenum*. (Image by U. Kück.) (D) Apothecium from *Pyronema confluens* (adapted from reference 446). Panels A and B show scanning electron micrographs, and panels C and D show light microscopic images. Arrows in panels A and B indicate ascospores that are actively discharged from the *S. macrospora* perithecium, while ascospores from *A. nidulans* are distributed when the mature fruiting body disintegrates.

haploid cell. In the upper cell, the two nuclei undergo karyogamy to form a diploid nucleus. This is the prerequisite for meiosis and a single postmeiotic mitosis. The eight nuclei are the origin of an eight-spored ascus, where spores have a linear order. Crozier formation occurs several times with the hymenium; thus, every perithecium may contain more than a hundred asci.

S. macrospora is closely related to *N. crassa*, and thus, cellular processes during the sexual cycle are very similar. However, *S. macrospora*, like *A. nidulans*, is a homothallic fungus. Thus, no mating-type type strains exist, and the sexual cycle starts by selfing. During this period, primordia (protoperithecia) are generated, which turn into mature flask-shaped perithecia after about 4 days (Fig. 9). For entry into the sexual cycle, no specific physiological stimuli are necessary, but biotin significantly promotes the formation of mature fruiting bodies (336). *S. macrospora* is a favorable object to study fruiting body development because it lacks asexual spores (337), and therefore, there is no interference between two different developmental programs.

Early on, both *N. crassa* and *S. macrospora* were used for differential gene expression studies, using expressed sequence tag (EST) sequencing or differential hybridization techniques to discover fruiting body-specific genes (338, 339). Since the isolation of ascomycete fruiting bodies is difficult due to their small size ($<$500 μm), in these studies, RNA was isolated not only from fruiting bodies but also from nonreproductive hyphae. A technical advancement occurred when laser microdissection was combined with transcriptome sequencing (RNA-seq) to avoid RNA isolation from nonreproductive

hyphae (340). This technique, first applied to *S. macrospora*, allowed the determination of gene expression patterns in reproductive and nonreproductive tissues.

By using laser microdissection, young fruiting bodies (protoperithecia) from *S. macrospora* were isolated for an extensive RNA-seq analysis, showing major differences in gene expression patterns between protoperithecia and the total mycelium. For example, in protoperithecia, 284 genes were upregulated and 2,305 genes were downregulated, compared to their expression in mycelia. Notable examples of upregulated genes are the pheromone precursor genes *ppg1* and *ppg2* (340).

A. nidulans changes developmental programs depending on external stimuli. Approximately one-fifth of the genes are differentially expressed when submerged vegetative hyphal cells are compared to cells during surface development (341). In an air interface, this fungus forms closed cleistothecia with sexual meiospores inside and also asexual conidia as mitospores, which are released into the air (Fig. 8 and 9). Sexual differentiation is favored in darkness and is linked to a specific secondary metabolism, whereas light promotes asexual spore formation (342). Snapshots of transcript and metabolite profiles during fungal development revealed that light significantly induces gene expression within 24 to 48 h after the transfer of liquid-grown mycelia to solid medium. Many light-induced genes are also expressed in the dark after a delay of up to 2 days. Darkness results in massive transcriptional reprogramming, inducing lipid-derived fungal pheromone synthesis during early sexual development and the expression of genes for cell wall degradation, presumably to mobilize energy for sexual differentiation (341, 343, 344).

Transcriptional expression is controlled by a well-orchestrated choreography of transcription factors (TFs) and signaling pathways. TFs encoded by MAT loci, directly involved in the control of sexual propagation, are the most intensively studied TFs (345). The MAT loci are found at identical genetic loci and represent idiomorphs (no alleles). They carry at least one gene encoding an α1-box-containing TF (MAT1-1-1) or a high-mobility-group (HMG) TF (MAT1-2-1) (346). While MAT loci from the Eurotiales usually carry a single TF-encoding gene, loci from the Sordariales usually have two or more genes for additional TFs. In the homothallic euascomycetes *F. graminearum* and *S. macrospora*, some, but not all, MAT locus-encoded TFs are required for the production of fertile perithecia (347, 348). A protein binding microarray analysis with the *F. graminearum* HMG TF domain (MAT1-2-1) revealed 25 target genes that are specifically required for sexual development (347). A recent genome-wide chromatin immunoprecipitation sequencing (ChIP-seq) analysis with the α1-box-containing TF (MAT1-1-1) from the heterothallic fungus *Penicillium chrysogenum* identified a DNA-binding motif that is highly conserved among euascomycetes and discovered several target genes directly involved in sexual development, such as *ppg1*, the homologue of the *S. cerevisiae* α-factor pheromone gene, and *pre1*, the ScSTE3 homologue (349).

Beside MAT-encoded TFs, other TFs were identified as having a distinct role in sexual propagation, including fruiting body formation. One intensively studied example is PRO1, a C_6Zn_2 TF with homology to the *S. cerevisiae* GAL4 TF (350). Microarray and RNA-seq analyses revealed PRO1-dependent gene expression in sexual and vegetative mycelia as well as in protoperithecia (351). RNA-seq analysis of laser microdissection-isolated protoperithecia from a *pro1* mutant showed that more than 400 genes were differentially regulated compared to wild-type protoperithecia (340). These results were compared with the results of a recent ChIP-seq analysis, which identified about 400 direct PRO1 targets (352). Among the differentially expressed genes detected in the RNA-seq analysis, there are 19 PRO1 target genes, such as those encoding the MAP kinases MAK1 and MEK1 or the adaptor protein HAM14. Similarly, ADV-1, a *N. crassa* PRO1 homologue, was recently found to have targets that are light and/or clock controlled as well as genes involved in fruiting body development (353). The Pro1 homologues RosA and NosA have negative and positive regulatory functions in *A. nidulans* sexual development (354, 355).

Recently, diverse posttranscriptional regulations and modifications were shown to markedly affect fruiting body formation. For example, the RNA interference (RNAi)

pathway controls sexual development in *N. crassa* (356), and a genome-wide survey in *F. graminearum* showed that the transcriptome is regulated by an exonic small interference RNA (exsiRNA)-mediated RNAi mechanism during ascospore formation (357). In the same ascomycete, several thousand A-to-I RNA-editing sites occur specifically in perithecia (358), and this process seems to be evolutionarily conserved, since it was also observed in sexual mycelia from *S. macrospora* and the apothecium-forming ascomycete *Pyronema confluens* (359). Interestingly, no RNA editing was found in sterile *S. macrospora* mutants, which form only immature young fruiting bodies (protoperithecia).

Another regulatory principle was discovered through the analysis of velvet domain proteins, which determine the ratio between asexual and sexual development in *A. nidulans*. The fungus-specific velvet domain is conserved in many filamentous fungi but is absent in yeasts and represents a dimerization and DNA-binding domain reminiscent of the mammalian NF-κB *rel* domain. Velvet domain proteins form homodimers as well as heterodimers and recognize hundreds of genomic DNA-binding sites (360). Velvet domain proteins affect not only sexual and asexual development but also spore viability in different organisms (361–364). *A. nidulans veA* was the first described velvet-domain-encoding gene (365), and its deletion results in the complete loss of sexual fruiting bodies (366).

Similar to mammalian *rel* proteins, the nuclear entry of the VeA protein is controlled, and translocation into the nucleus preferentially happens in darkness (367). VeA physically interacts with light regulators, such as the phytochrome FphA (368). Heterodimer formation between VeA and VelB is promoted by VeA phosphorylation by the MAP kinase MpkB (369). The VeA-VelB heterodimer forms a nuclear trimeric complex with the methyltransferase LaeA, which is required for sexual development and coordinated secondary metabolism (364). The loss of LaeA results in impaired secondary metabolism and a lack of the *Aspergillus*-specific sexual Hülle cell type, which supports fruiting body growth (370, 371). The absence of LaeA results directly or indirectly in increased repressive trimethylation marks at histone 3 in *A. nidulans* (372) or *Trichoderma atroviride* (373). VeA is a hub for several interacting methyltransferases, including LaeA, VipC/LlmB, VapB, or LlmF in *A. nidulans* or *P. chrysogenum* (374–376). The *A. nidulans* VipC-VapB methyltransferase heterodimer acts as negative regulator of sexual development and as a positive regulator of asexual development and is part of an epigenetic methyltransferase signal transduction pathway. The plasma membrane protein VapA binds VipC-VapB during sexual development. Release to the nucleus depends on environmental conditions where asexual development is favored. Nuclear VipC-VapB reduces negative histone tags and promotes asexual development (377).

Posttranslational modifications by ubiquitin-like proteins are also directly linked to fungal development. SUMO is required for sexual development in *A. nidulans*, where the COMPASS histone 3 lysine 4 methyltransferase complex connects the SUMO network to histone modifications (378). The ubiquitin-like protein Nedd8 controls various cullin E3 ubiquitin ligases and the incorporation of F-box proteins as substrate receptors, which is important to control the ratio between sexual and asexual differentiation (379–381). The multiprotein complex COP9 signalosome represents a deneddylase, which removes Nedd8 and is involved in the coordination of sexual development and secondary metabolism. The defects in genes for COP9 subunits are genetically epistatic to mutations in genes for velvet domain proteins in *A. nidulans* (382–384), but the exact molecular mechanism of velvet domain protein stability control is as yet unknown (385, 386). DenA represents a second deneddylase, which interacts at septa with the DenA-interacting protein DipA. This protein controls septum positioning and asexual spore formation (387). F-box proteins involved in fungal development include GrrA, which is required during late fruiting body formation for ascospore maturation (388), or Fbx15, which is required for asexual and sexual development in *A. nidulans* (389) or for virulence in *A. fumigatus* (390).

Another multisubunit complex that regulates fruiting body development posttranslationally is the striatin-interacting phosphatase and kinase (STRIPAK) complex (391,

392). It is defined by at least six core subunits, which have structural homologues in fungi and animals. In *S. macrospora* and *N. crassa*, the deletion of genes for subunits of the STRIPAK complex results in sterile strains, which generate only protoperithecia or lack any sexual structures (393–395). Evidence from diverse systems suggests that the STRIPAK complex coordinates the phosphorylation and dephosphorylation of target proteins and thus controls diverse cellular programs. It is suggested that the regulatory, catalytic, and scaffolding subunits of the phosphatase PP2AA, all of which belong to the core of the STRIPAK complex, are relevant for the dephosphorylation process (396). However, the precise mechanisms of the regulation of phosphorylation are so far unknown, and the kinases involved remain to be identified for most fungi (397). It is tempting to speculate that STRIPAK complex transfers phosphorylation signals to other fungal signaling pathways, since the septation initiation network (SIN), the target of rapamycin 2 (TORC2) pathway, and the cell wall integrity (CWI) pathway were shown to be connected with the STRIPAK complex (392). Future investigations will try to understand how this complex is assembled and regulated to control fungal cellular development.

HYPHAL DIFFERENTIATION IN RESPONSE TO A HOST OR A PREDATOR

Dimorphism and Pathogenicity

Dimorphism is the capacity of fungi to change their morphology during their life cycle from yeast-like growth to filamentous growth. For a variety of fungi that are pathogenic to mammals, plants, or insects, the dimorphic shift between hyphae and yeast is critical for pathogenesis. The change in morphology is typically triggered by multiple signals and is controlled by complex signaling pathways that ensure the integration of the altered developmental program in the cell and the physiological modifications that facilitate interactions with the host. For the causal agent of Dutch elm disease, *Ceratocystis ulmi*, the yeast cells disperse within the infected tree passively within the xylem sap flow, while the invasion of adjacent, uninfected xylem vessels requires the hyphal form (398). Similarly, for the human pathogen *Candida albicans*, the hyphae predominate at the primary site of infection in tissues, while yeast cells are found more on the cell surface of epithelial cells or are formed from penetrating hyphae (reviewed in reference 399). Yeast-mycelium dimorphism is also a feature of the well-studied plant pathogen *Ustilago maydis*, a basidiomycete that infects maize plants. The haploid, yeast-like sporidia divide by budding and grow strictly saprophytically. The infectious dikaryotic hyphae are formed by the fusion of two sporidia; these hyphae are not able to grow saprophytically but depend strictly on the host plant for development and for the completion of the sexual cycle (for a review, see reference 400).

The fusion of compatible sporidia is controlled by two independent mating-type loci, termed the *a* and *b* loci; the *a* locus mediates cell fusion, while the *b* locus controls the formation of the dikaryotic filament, plant infection, and sexual development. The *a* locus encodes a pheromone/receptor system; upon the binding of a peptide pheromone (encoded by the *mfa* genes) to the compatible seven-transmembrane receptor (encoded by *pra*), a conserved MAPK module is activated, which leads to the direct phosphorylation of the pheromone response factor Prf1, a HMG transcription factor with pivotal function for mating and cell fusion. Prf1 triggers the expression of a defined set of genes by binding to a pheromone response element (*pre*), including *mfa* and *pra*, which ultimately leads to the formation of short filamentous conjugation tubes that grow to each other and fuse. Prf1 appears to function as a platform to integrate different signals: (i) by phosphorylation via the pheromone signal-responsive MAPK cascade; (ii) by phosphorylation via a cAMP-dependent protein kinase (PKA) that probably integrates environmental signals, such as nutrient availability or surface hydrophobicity; and (iii) by transcriptional regulation via Rop1 and Hap2 that converge additional signals on *prf1* gene expression (reviewed in references 170, 400, and 401).

Prf1 is required for the expression of the *b* mating-type genes *bE* and *bW*, which encode two homeodomain transcription factors. Regulation of the *a* and *b* mating-type pathways via Prf1 depends on its phosphorylation status: PKA signaling is required for

both pathways, while MAPK signaling has no influence on *a* gene expression but is required for *a*-mediated cell cycle arrest and *b* gene expression (402).

After cell fusion, the bE and bW proteins form a heterodimeric complex but only when they originate from different alleles of the multiallelic *b* locus (e.g., bE1 and bW2). The filaments formed upon the activation of the *b* pathway grow unipolarly, but only the tip compartment is filled with cytoplasm and contains the two nuclei that remain arrested in the G_2 phase; all other distal compartments appear highly vacuolized. Similarly to other pathogenic fungi, entry into the host cell is facilitated by a specialized structure of hyphae called the appressorium. Unlike the dome-shaped melanized appressoria of *Magnaporthe oryzae* or *Colletotrichum* species, which generate high turgor pressures to penetrate the plant cuticle by "brute force" (403, 404), the appressoria of *U. maydis* are nonmelanized and thought to penetrate the plant cuticle via the secretion of plant cell wall-degrading enzymes (CWDEs) (405).

Upon penetration of the plant cuticle, the cell cycle block of the hyphae is released, and fungal cells start to divide, which is accompanied by the formation of clamp connections, structures typical for basidiomycetes that facilitate the proper distribution of the two nuclei during cell division.

During the morphological and physiological differentiation of the hyphae, the bE/bW heterodimer orchestrates a hierarchic, multilayered transcriptional network regulating more than 340 genes. As expected, bE/bW-responsive genes can be classified into functional categories such as "cell cycle," "DNA metabolic process," "cytoskeleton," and "microtubule cytoskeleton," attributed to the transition from budding to polarized growth and cell cycle arrest (406). Among the downregulated genes are, for example, the *cln1*, *clb1*, and *clb2* genes, which encode a G_1-type cyclin and two B-type cyclins. Other genes have potential functions in cell wall synthesis and modification, such as chitin synthases, exochitinases, chitin deacetylases, and exo- and endoglucanases, indicating that during the switch from budding to filamentous growth, the cell wall composition is altered (406).

The central node within the *b*-regulatory network is the zinc finger transcription factor Rbf1 (required for *b*-dependent filamentation), a direct target gene of the bE/bW heterodimer (Fig. 10). Rbf1 is both required and sufficient for filament formation; the induction of the *rbf1* gene in a Δ*b* strain leads to hyphae that are indistinguishable from *b*-induced hyphae. Rbf1 is required for the regulation of the majority (334 out of 345) of *b*-regulated genes (406). One of the few genes regulated directly by bE/bW is *clp1*, encoding a protein specific for basidiomycetes without domains with assigned functions. In *Coprinopsis cinerea*, Clp1 is required for clamp cell formation (407), and similarly, in *U. maydis* Δ*clp1* hyphae, proliferation within the plant is stalled, and no clamp cells are formed (408). Clp1 interacts with both the bW protein and Rbf1; the interaction with bW renders the bE/bW complex inactive, while the interaction with Rbf1 results in a specific downregulation of the pheromone pathway (409). As both pathways independently activate polarized growth and cell cycle arrest, Clp1 expression inhibits filamentous growth and allows the release of *a*- and *b*-induced cell cycle arrest. Although the *clp1* transcript is detectable immediately after *b* induction, the protein is evident only when the hyphae penetrate the plant. At this point, the transcriptional rewiring of the filament is connected to a rather unexpected pathway, the unfolded protein response pathway. This pathway is a conserved eukaryotic signaling pathway that regulates endoplasmic reticulum homeostasis during stress, which may result from an increased demand for protein secretion. The interaction of Clp1 with the central transcriptional regulator of the unfolded protein response pathway in *U. maydis*, Cib1 (Hac1 in *S. cerevisiae*), leads to the stabilization of Clp1. In addition, the activation of Cib1 leads to the Clp1-independent downregulation of bE/bW expression, a second mechanism that reduces filamentation. Apparently, the cross talk between the unfolded protein response pathway and the *b*-regulated developmental program adapts developmental progression and increased secretion of proteins required during the plant infection process (409, 410).

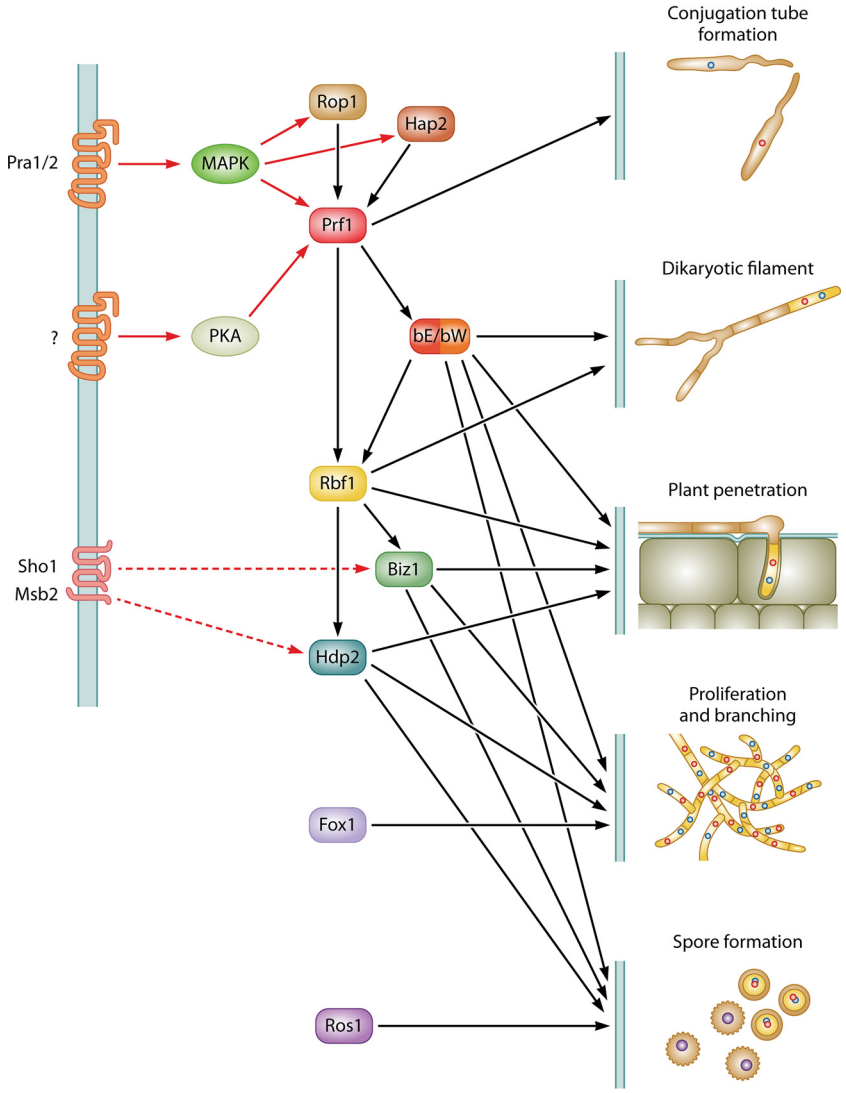

FIG 10 Hierarchical regulatory network controlling different cell types of *Ustilago maydis*. During the fusion of two nonpathogenic, yeast-like sporidia, the binding of peptide pheromones to the pheromone receptor (Pra) activates a MAP kinase (MAPK) cascade, and environmental signals feed via a so-far-unidentified receptor into a cAMP-dependent protein kinase (PKA) pathway. Both pathways converge at Prf1, the central transcription factor required for mating-dependent gene expression. Prf1 is additionally regulated at the transcriptional level by the transcription factors Rop1 and Hap2, both of which are thought to be targets of the MAPK cascade. The phosphorylation of Prf1 results in the induction of the *bE* and *bW* genes; after the fusion of two sporidia, bE and bW form a heterodimeric transcription factor that is the master regulator to induce filamentous growth and pathogenic development. The central node for gene regulation at the onset of pathogenic development is the transcription factor Rbf1. *rbf1* gene expression is already induced by Prf1 prior to cell fusion, but expression is boosted after cell fusion and the formation of the filamentous dikaryon by the bE/bW heterodimer. Rbf1 is sufficient to trigger the expression of all genes required for plant penetration. Among the Rbf1-induced genes are the transcription factors Biz1 and Hdp2, both of which are absolutely required for plant infection. Hdp2 and Biz1 are highly expressed at the later stages of *in planta* development, while *rbf1* expression is barely detectable within the plant. *biz1* and *hdp2* are also regulated by physical cues on the plant surface via the plasma membrane receptor Sho1/Msb2. Two transcription factors specifically expressed during the biotrophic phase are Fox1, which is thought to contribute to the regulation of several effector genes, and Ros1, a regulator for the late developmental steps during spore formation.

The interaction of Clp1 with both Rbf1 and bW sets the time for the release of the G_2 cell cycle at the initial stage of plant penetration. At this particular point, the cell cycle has to be tightly controlled: for the formation of the appressoria, G_2 arrest is required (411), but for subsequent hyphal differentiation, the G_2 block has to be released. A similar tight link between appressorium formation and the cell cycle has

been described for *M. oryzae*, where nuclei have to undergo a single round of replication before appressoria are formed (412, 413). In *U. maydis*, the two Rbf1-dependent transcription factors Biz1 and Hdp2 direct the *b* transcriptional network toward the formation of appressoria. Neither one of them influences filament formation *per se*, but their deletion leads to drastically reduced or abolished appressorium formation, respectively (414, 415). The two transcription factors are thought to implement signals from the host plant to the developmental program, as the expression of both genes is influenced via the surface cue sensor Sho1/Msb2 (416). It is conceivable that Hdp2 and Biz1 maintain the cell cycle arrest required for appressorium formation, as the induction of both factors causes cell cycle arrest, which is achieved via the downregulation of key cell cycle regulators, as for the mitotic cyclin Clb1 in the case of Biz1 (414).

For each of the components of the *b*-mediated signaling cascade mentioned so far, their independent deletion leads to a complete loss of virulence already at the stage of plant penetration. Accordingly, their function for later stages of fungal development within the plant is hard to determine. Only for the bE/bW heterodimeric complex itself has it been shown by means of a temperature-sensitive bE allele that bE/bW is required for all stages within the plant: the shift to the restrictive temperature stalls development immediately, cells fail to form polarized structures, and bulbous, round cells that contain multiple nuclei are formed instead (417). Both Hdp1 and Biz1 are expressed during the biotrophic stage. Global gene expression studies upon the ectopic expression of Biz1 and Hdp2 show a significant overlap of genes that are specifically induced during plant penetration but also during later stages, indicating that the two transcription factors might have a pivotal function for biotrophic development (M. Vranes and J. Kämper, unpublished data). Rbf1 expression is sufficient to initiate pathogenic development in a strain deleted for *b*; when combined with an ectopically expressed *clp1* gene, the hyphae undergo some mitotic divisions even before hyphal development is stalled (409). However, the expression of *rbf1* appears to be limited to the very early stages of plant infection; at later stages, the transcript is not detectable, indicating that the expression of *rbf1*-dependent transcription factors must be rewired via an additional, plant-specific pathway (M. Vranes, M. Jurca, and J. Kämper, unpublished data). It is conceivable that additional regulators are required for the fungus to adapt to the specific environment within the plant, to different tissues, and to changing metabolic conditions. One of the transcription factors specifically induced after the penetration of the plant is the forkhead protein Fox1. The deletion of *fox1* leads to attenuated virulence and increased host defense symptoms, which might be attributed to the Fox1-dependent regulation of several effector genes (418). The master regulator for the later stages of *in planta* proliferation was recently identified: Ros1 is not required for plant colonization or for the formation of tumors but is essential for karyogamy and subsequent spore formation. Ros1 influences the expression of about 30% of all *U. maydis* genes, and ChIP-seq analysis revealed that 40% of these genes might be directly regulated by Ros1, among which are a large number of *b*-regulated genes. Apparently, Ros1 counteracts the *b* developmental program to shift the cells into the next developmental stage within the *U. maydis* life cycle, the formation of diploid spores (419).

Injury Induces Development

The wound response is a crucial process for the survival of multicellular organisms. Plants, as sessile organisms, cannot escape from injuries caused by chewing insects or larger herbivores, whereas animals are exposed to mechanical damage and injuries caused mainly by predators. Consequently, plants have developed mechanisms to rapidly respond to wounding. Like plants, due to their absorptive nutrition mode and their immobility, multicellular (filamentous) fungi are preys to a variety of animal predators, including fungivorous nematodes and insects.

In regenerative animals, wounding can trigger the regrowth of a missing body part, involving gene expression changes specific for tissue regeneration (420–422). Similarly,

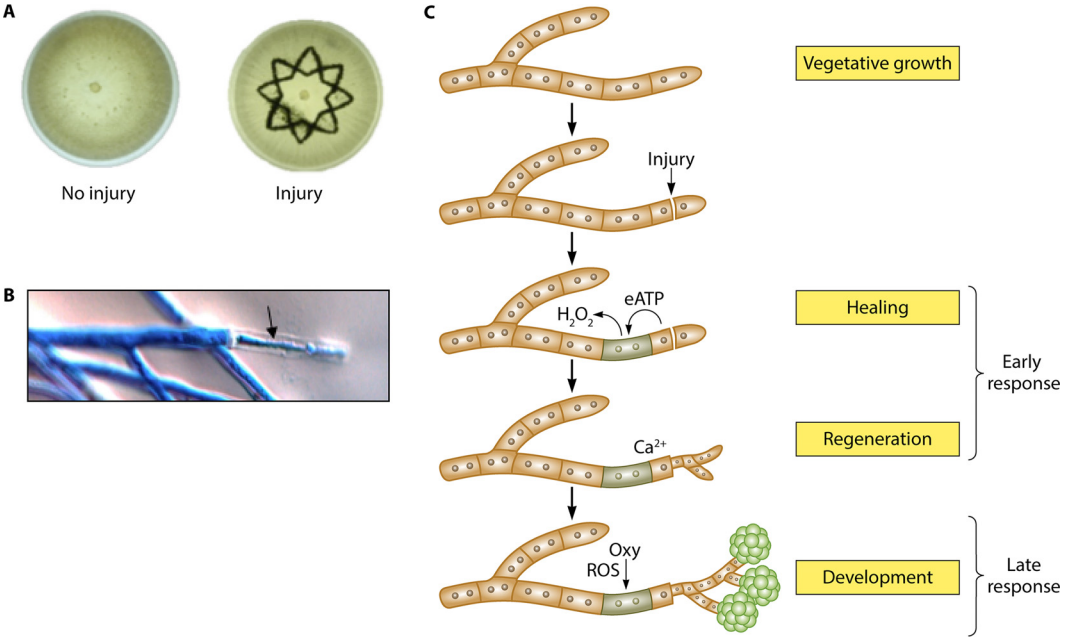

FIG 11 The injury response in *Trichoderma*. (A) A colony of *T. atroviride* (IMI206040) growing in the dark was damaged with a cookie mold and photographed 48 h later (right). An undamaged control is also shown (left). (B) Microscopic changes observed upon injury. One hour after injury, hyphae were stained with lactophenol cotton blue and examined under a light microscope. Arrows indicate newly formed hypha. (C) Illustration of the regeneration and conidiation processes of *T. atroviride* after injury. Upon damage, ATP is released (exogenous ATP [eATP]), triggering an increase in the level of cytosolic calcium required for regeneration (E. Medina-Castellanos, J. M. Villalobos-Escobedo, M. Riquelme, N. D. Read, C. Abreu-Goodger, and A. Herrera-Estrella, unpublished data). ROS and oxylipins (Oxy) play an important role in cell differentiation during conidiophore formation in response to injury. (Adapted from reference 426.)

plant and moss cells can be reprogrammed to initiate tip growth in wounded tissues (423).

Filamentous fungi rapidly respond to injury by sealing their septal pores using Woronin bodies in ascomycetes and septal pore caps in basidiomycetes, which prevent the leakage of cytoplasmic contents and prevent cell death (424, 425). In ascomycetes, sealing of the septal pore is followed by the formation of one or more hyphal tips at the plugged septum, resulting in the reinitiation of polar growth and hyphal reconnection (424). Nevertheless, a number of fungal species, such as the basidiomycetes *Schizophyllum commune* and *Sclerotium rolfsii* and the ascomycetes *Trichoderma atroviride* and *Aspergillus flavus*, respond to injury by producing reproductive structures. *S. commune* produces fruiting bodies in the damaged area (426), while in *S. rolfsii*, mechanical damage induces sclerotium formation (427), and in *A. flavus* and several species of *Trichoderma*, it triggers the production of conidia (301, 426).

When a *T. atroviride* colony is wounded, the first morphological change observed is the regeneration of the damaged hyphae with the formation of "new," significantly thinner, hyphal tips 1 h after injury. After 24 h, the formation of phialides is observed, and 48 h later, conidiophores with mature spores have formed exclusively from the new hyphae (Fig. 11). This phenomenon is perhaps the best-studied response to wounding at the molecular level, where RNA-seq experiments led to the identification of 933 differentially expressed genes that respond within minutes (301). Among the early-induced genes (15 to 30 min), the presence of genes encoding proteins involved in calcium signaling and transport, redox balance, stress responses, the cell cycle, and cell death, as well as transcription factors, should be highlighted. Late-induced genes (30 to 60 min) encoded mainly proteins involved in the DNA damage response and the cell cycle and proteins with oxide reductase activity.

Among the injury-responsive genes, at least 25 were related to cytoskeletal organization, DNA replication, and the cell cycle, including the *sda1* gene, which plays a

critical role in the passage of cells arrested in G_0 phase into the G_1 reinitiation of the cell cycle (428), suggesting that these genes might play an important role in the regeneration of damaged hyphae. At least 60 genes encoding proteins related to redox reactions were differentially expressed, and 16 genes encoding ROS-scavenging proteins were strongly repressed at short times after injury, but their levels began to increase a few minutes later to finally reach those observed in noninjured controls or to reach even higher levels. This suggests that the cell initially allows the generation of oxidative stress upon injury.

A noteworthy observation was the induction of a series of genes considered key components in calcium signaling in response to different types of stress (429), including calcium transporters, phospholipase C, Ca^{2+}/calmodulin-dependent kinase 1 (CAMK-1), and a homologue of the transcription factor Crz1, which regulates responses to calcium and is essential for membrane integrity and oxidative stress responses (430). These data suggested that calcium signaling could play an important role in the injury response of *T. atroviride*. Consistent with this, in plants and animals, the injury response involves increased intracellular calcium levels and the activation of the calcium signaling machinery (431). There is also good evidence that Ca^{2+} plays a crucial role in polarized growth. It was shown that the concentration of Ca^{2+} oscillates during polar hyphal extension and that this oscillation may be responsible for oscillations of vesicle secretion and, thus, the oscillatory growth of hyphae. Because stress responses cause dramatic changes in intracellular Ca^{2+} concentrations, effects of stress on polar cell extension are likely (76).

Oxylipin production also plays an important role in the response to injury of plants and animals, mediating wound responses (432). A *T. atroviride* transcriptome analysis indicated that genes encoding a lipoxygenase, a cytochrome P450, and a 12-oxophytodienoate reductase were induced. Interestingly, transcriptome analysis of a *T. atroviride* Δ*noxR* mutant, which fails to develop conidiophores in response to injury, showed that all genes related to oxylipin biosynthesis were either repressed or not responsive to injury, suggesting that oxylipin biosynthesis is compromised in a Δ*noxR* mutant background. In this regard, as mentioned above, oxylipins are known to regulate the conidiation of *A. nidulans* and *Penicillium chrysogenum* (433, 434) and might serve as signaling molecules in the response of *T. atroviride* to injury.

In plants and animals, high levels of ROS and intracellular Ca^{2+} provoke the death of damaged cells (435, 436). For fungi, it has also been reported that elevated levels of ROS induce programmed cell death (PCD) (437), and the generation of ROS depends on Nox activity (438). For ascomycetes, it has been reported that oxidative stress can induce differentiation (295, 439). In particular, various reports demonstrated that NoxA and NoxR are involved in sexual development in fungi (296, 298). In *T. atroviride*, the regeneration of injured hyphae is not affected by the lack of either *nox1* or *noxR*, whereas conidiophore development is severely affected, suggesting that at least two signaling pathways are activated by mechanical injury.

Recent high-throughput RNA-seq analyses of the basidiomycete *Coprinopsis cinerea* challenged with the fungivorous nematode *Aphelenchus avenae* revealed the specific induction of 20 genes, some encoding previously characterized nematotoxic lectins (440, 441). Challenge of the mycelium with *A. avenae* also led to the induction of a small set of genes encoding putative antibacterial proteins. Some of these genes were also induced upon challenge of the mycelium with *Escherichia coli* and *Bacillus subtilis* (441). Surprisingly, mechanical damage resulted in only 10 differentially expressed genes, and none of them corresponded to those differentially expressed when the fungus was challenged with the fungivorous nematode. These results suggest not only that fungi have the ability to induce specific innate defense responses similarly to plants and animals but also that the nematode might have the ability to block the response to mechanical damage.

Although inducible defense strategies in plants attacked by herbivorous insects are well known, induced resistance of fungi to fungivorous animals is largely unknown. The production of toxic secondary metabolites is thought to mediate resistance to fungi-

vory. However, whether fungi change their pattern of secondary metabolite production to increase resistance to fungivory remained an open question until recently, when Döll and coworkers (442) demonstrated that grazing by a soil arthropod, *Folsomia candida*, on *A. nidulans* induced a phenotype that repelled subsequent attacks and retarded fungivore growth. Arthropod-exposed colonies produced significantly larger amounts of toxic secondary metabolites and invested more in sexual reproduction than did unchallenged fungi. Their results indicated that in *A. nidulans*, fungivore grazing triggered the coregulated allocation of resources to sexual reproduction and chemical defense (442). That same group established a mechanism of regulation through RsmA, a recently discovered YAP-like bZIP protein that impacts secondary metabolite production through the regulation of the C6 transcription factor *aflR*. The fungivore *Folsomia candida* preferred feeding on wild-type *A. nidulans* rather than on an *OE*::*rsmA* strain, indicating that RsmA may have a critical function in mediating direct chemical resistance against predation (443). Posterior studies provided additional evidence that insect grazing is capable of inducing resistance to further grazing in *A. nidulans*. Such a phenotypic shift in resistance to fungivory was accompanied by the upregulation of genes involved in signal transduction (*mpkB*), epigenetic regulation (*laeA*), and secondary metabolite biosynthesis pathways (*ppoA* and *stcA*) and of the transcription factors *aflR* and *rsmA*.

As described above, fungi show transcriptional responses to both mechanical damage and fungivory by chewing predators. Nevertheless, the signals that warn fungal cells of this danger remain largely unknown, although it was recently proposed that extracellular ATP and extracellular Ca^{2+} could serve as danger-associated molecular patterns, triggering the response (444).

CONCLUDING REMARKS

This review highlights similarities and discrepancies found at the molecular and cellular levels in the cell morphology and differentiation of a few selected fungal species that are subjects of study in our laboratories. Comparative studies have shown us how some mechanisms that are apparently conserved at the molecular level can behave very differently at the cellular level in different species that are relatively close phylogenetically.

While an extraordinary amount of knowledge has been acquired in the field of fungal cell biology in the last 2 decades, some basic questions remain essentially unanswered. For instance, what delimits the size and growth rate of hyphae in different fungal species? What determines the different conidiation patterns or sexual structures? What are the molecular factors underlying distinct differentiation processes, and how did they evolve? These are some of our challenges for years to come.

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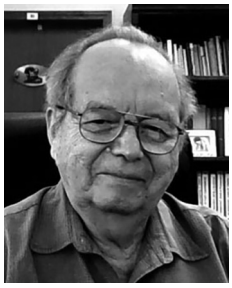
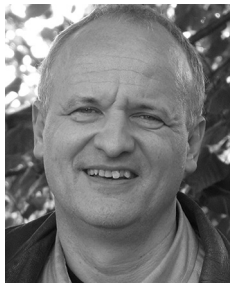
Meritxell Riquelme obtained her Biology degree at the University of Barcelona, Spain. After completion of an M.Sc. in Plant Pathology and a Ph.D. in Microbiology at the University of California, Riverside, she was a postdoctoral fellow at the University of Oxford, United Kingdom, where she investigated the mating-type genes in the mushroom *Coprinopsis cinerea*. In 2004, she joined the Department of Microbiology at the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Baja California, Mexico, where she is a Research Professor and Department chair. From 2012 to 2013, she was visiting Professor at the Department of Cellular and Molecular Medicine at the University of California, San Diego. Her research interests include the secretory routes of cell wall-building vesicles involved in hyphal morphogenesis in *Neurospora crassa*. Also, she studies fungi of extreme ecosystems, including semiarid regions of Baja California and deep-sea sediments of the Gulf of Mexico.

Jesús Aguirre obtained his Biology and Biomedical Research Ph.D. degrees from the Universidad Nacional Autónoma de México (UNAM). As a postdoctoral associate at the Department of Genetics, University of Georgia, he studied the genetic regulation of *Aspergillus nidulans* asexual development. Since 1991, he has been a researcher at the Instituto de Fisiología Celular-UNAM. After proposing cell differentiation as a response to a hyperoxidant state, his research has been focused on studying the mechanisms by which fungi produce, perceive, and detoxify reactive oxygen species (ROS) and the roles that ROS have in gene expression and cell differentiation.



Salomon Bartnicki-García (Ph.D. 1961) is a Professor Emeritus of the Plant Pathology Department of the University of California, Riverside. In 2000, he became a member of the Ensenada Center for Scientific Research and Higher Education (CICESE), first director of the new Division of Experimental and Applied Biology, and Investigator in its Microbiology Department. In his long career, he covered multiple aspects of the physiology and biochemistry of fungi. He analyzed the chemistry of cell walls and developed a phylogenetic scheme supporting the existence of two different evolutionary lines. His studies of chitin synthesis yielded the first evidence of microfibril assembly *in vitro*, a preamble to the discovery of chitosomes, the carriers of chitin synthase. A computer simulation of fungal growth led to the vesicle supply center concept, which explained the function of the Spitzenkörper. He was a cofounder of the journal *Experimental Mycology* and an author of 158 research and review articles.



Gerhard H. Braus studied Biology at the University of Freiburg in Germany. He earned a Ph.D. degree and habilitation at the ETH Zürich in Switzerland. He was an Associate Professor of Biochemistry at the University of Erlangen and is currently Full Professor of Microbiology and Genetics in Göttingen, Germany. Additional periods included the Biocenter in Basel; the University of Georgia in Athens, GA; and the BMS Institute of Functional Genomics, Princeton, NJ. His research focus is on yeasts and filamentous fungi and includes the interplay between development (adhesion, filament formation, and tissue formation) and secondary metabolism, the impact of fungal pathogens on human health and agriculture, and fungi as models for neurodegenerative diseases (Morbus Parkinson).

Michael Feldbrügge studied Biology at the University of Cologne. He obtained his Ph.D. thesis at the Max Planck Institute for Plant Breeding in Cologne, working on transcriptional regulation in plants. As a postdoctoral fellow, he investigated plant mRNA stability at the Plant Research Lab in East Lansing, MI. For a second postdoctoral research project, he joined the Institute for Genetics and Microbiology at the Ludwig Maximillians University in Munich, working on signaling in *Ustilago maydis*. He switched as a group leader to the Max Planck Institute for Terrestrial Microbiology in Marburg, working on fungal RNA biology. He is now full professor at the Heinrich Heine University in Düsseldorf and head of the Institute for Microbiology. His research interests are microbial cell biology, pathogenicity, and biotechnology. He is a member of the Cluster of Excellence on Plant Sciences (CEPLAS) and the Bioeconomy Science Center (BioSC) of North Rhine-Westphalia.

Ursula Fleig obtained her B.A. in molecular genetics at Trinity College, Dublin, Ireland, and her Ph.D. at the Biocenter, University of Basel, Switzerland. She then went to the laboratory of Prof. Paul Nurse at the University of Oxford, UK, to investigate cell cycle control mechanisms in the model yeast *Schizosaccharomyces pombe*. After working as a research associate at the University of Vanderbilt, Nashville, TN; the Max Planck Institute, Cologne, Germany; and the University of Giessen, Giessen, Germany, she is now a Professor at the Heinrich Heine University, Düsseldorf, Germany. *S. pombe* has remained her favorite research organism, which is used to understand how eukaryotic cells control chromosome transmission fidelity and cell morphogenesis.

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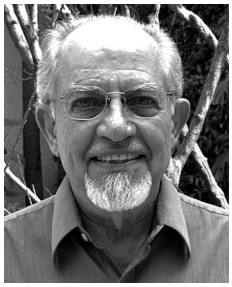


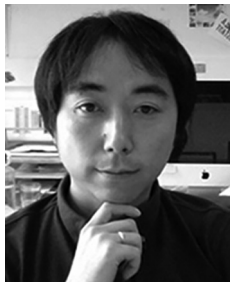
Wilhelm Hansberg obtained his M.D. degree at the Universidad Nacional Autónoma de México (UNAM) and thereafter obtained an M.S. and a Ph.D. in Biochemistry at the Faculty of Chemistry, UNAM, studying cell differentiation in chicken reticulocytes and thereafter studying *Xenopus* oogenesis at the Consiglio Nazionale delle Ricerche, Rome, Italy. He is now Professor at the Instituto de Fisiología Celular, UNAM, where he investigates cell differentiation in microorganisms as a response to oxidative stress, using mainly *Neurospora* conidiation as a model system. He made a two-year leave at the Heinrich Heine University, Düsseldorf, Germany, studying reactive oxygen species in *Neurospora*. He is also interested in the structure-function relationship of antioxidant enzymes, mainly catalases, using crystallography and molecular dynamics and in mathematical modeling of cell differentiation.

Alfredo Herrera-Estrella obtained his Ph.D. from the State University of Ghent in Belgium, working under the direction of Prof. Marc Van Montagu. He described for the first time *Agrobacterium* virulence proteins capable of carrying transfer DNA (T-DNA) into the plant cell nucleus. Prof. Herrera-Estrella pioneered the development of molecular tools for the study of the biocontrol agent *Trichoderma atroviride*. He continued those studies at the Irapuato Unit of the Center for Research and Advanced Studies, where he started working on the mechanisms involved in light perception in *Trichoderma*. In 2000, he was awarded the prize of the Mexican Academy of Sciences. By 2004, he became involved in the establishment of the National Laboratory of Genomics for Biodiversity in Mexico. Since then, his group has been involved in functional genomics projects in crop plants and fungi. He now continues his efforts towards the elucidation of signaling cascades triggering asexual development in fungi.

Jörg Kämper studied Biology at the University of Bochum in Germany and obtained his Ph.D. working with linear plasmids in yeasts. As a postdoctoral fellow at the Ohio State University, he started to work with pathogenic fungi (*Fusarium*); since 1990, he has worked with the plant-pathogenic basidiomycete *Ustilago maydis* as a model, first at the University of Munich and continuing at the Max Planck Institute in Marburg, Germany. He is now a Research Professor and head of the Department of Genetics at the Institute of Applied Biosciences at the Karlsruhe Institute of Technology (KIT). His research focuses primarily on the regulatory network during the switch from the saprophytic to pathogenic lifestyles of *U. maydis* and the adaptation of hyphae to the environment in the host plant. Other interests are connections between the RNA splicing machinery and cellular transport processes.

Ulrich Kück is a Professor of Molecular Botany at Ruhr University, Bochum (RUB). After completion of a Ph.D. at RUB with Karl Esser and a postdoctoral fellowship at Harvard University with Lawrence Bogorad, he completed his Habilitation in 1986. He became Associate Professor at Frankfurt University and has held the chair for Molecular Botany at RUB since 1991. He served as a Dean at the biological and biotechnical faculty and was spokesman of a DFG-funded research initiative for eight years. In 2009, he became principal investigator of a Christian Doppler Laboratory for fungal biotechnology. Currently, he is a member of the review board of the German Science Foundation and a fellow of the American Academy of Microbiology. His research addresses fundamental and applied aspects of gene expression in ascomycetes. His current research interests range from investigating eukaryotic signaling pathways controlling fungal sexual development to the regulation of secondary metabolism in filamentous fungi.

Rosa R. Mouriño-Pérez obtained an M.D. degree at the Universidad Nacional Autónoma de México (UNAM) and a doctor in science degree in Marine Ecology at the Center for Scientific Research and Higher Education of Ensenada (CICESE). She was a postdoctoral researcher at the Scripps Oceanography Institute of the University of California, San Diego. Currently, she is a full Research Professor in the Department of Microbiology at CICESE and head of the Advanced Microscopy Unit at the same institution. Her research has been oriented toward the understanding of the structure, organization, and function of microtubules and actin cytoskeletons and their associated proteins and their association with endocytosis and polarized growth of fungal cells of *Neurospora crassa*. She is also studying the diversity of *Candida* spp., risk factors for women and HIV/AIDS patients, and antifungal drug resistance in Baja California.



Norio Takeshita obtained his Ph.D. in Microbiology at the University of Tokyo, Japan. As a Humboldt foundation postdoc fellow, a research associate, and a group leader at the Karlsruhe Institute of Technology, Germany, he investigated the molecular mechanisms of polarized tip growth of filamentous fungi by up-to-date fluorescent microscopy, including superresolution imaging. He is now an Assistant Professor at the University of Tsukuba, Japan. His current research interests include microbe interactions and roles in ecology.

Reinhard Fischer studied Biology at the University of Marburg, Germany, and graduated with work on archaea. He then moved as a postdoctoral fellow to the University of Georgia, Athens, GA, to work on the molecular biology of fungal development. He returned afterwards to the University of Marburg and the Max Planck Institute for Terrestrial Microbiology in Marburg, where he established his own research group on fungal cell biology. He was appointed to the University of Karlsruhe, Germany, in 2004 and is now full professor at the Karlsruhe Institute of Technology (KIT). His research is focused on the analysis of polar growth of *Aspergillus nidulans*, on light signaling in *A. nidulans*, and on the analysis of the secondary metabolism of *Alternaria alternata*.