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The evolution of fungal morphogenesis, a personal account

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Abstract: This article describes the evolution of the field of fungal morphogenesis, its beginning at the end of the 19th century and its exponential growth during the second half of the 20th century, continuing until the present day. The main theme correlates biological progress with the advent of new technologies. Accordingly the article describes the discovery of apical growth, the fibrillar nature of the fungal wall, the chemistry of the cell wall, the search for biochemical pathways in morphogenesis, the discovery of the Spitzenkörper, the apical gradient of wall synthesis, key highlights in ultrastructural research, the development of mathematical models particularly the vesicle supply center (VSC) model, the revolution brought about by molecular biology and unique discoveries such as the hydrophobins and γ-tubulin and some the latest triumphs of the marriage between molecular genetics and confocal microscopy. Credit is given to the investigators responsible for all the advances.

Key words: evolution, history, hyphal fungi, morphogenesis, polarized growth

The beginning

The question of how fungal cells attain their shape has been an increasingly attractive topic for investigation for more than a century. What eventually would be called fungal morphogenesis had an august beginning with the pioneering work of the German botanist Otto Reinhardt who discovered in 1892 that the amazingly fast growth of fungal colonies originated in the apices of their hyphae. With an unsophisticated bright field microscope and the clever use of cartographic projections Reinhardt not only discovered apical growth but offered a plausible explanation of how the hemispherical or hemiellipsoidal tips are converted continuously into cylindrical tubes (Reinhardt 1892). Reinhardt was clearly ahead of his time and for decades there was hardly any mention of his discovery. It was not until the second half of the 20th century that hyphal morphogenesis became a popular subject for investigation.

The first half of the 20th century brought some momentous advances in fungal biology; for instance, A.H. Reginald Buller's (1931) graphic analysis of the genesis of a colony of *Coprinus*; Edward Castle's (1936, 1938) analysis of the spiral growth and phototropism of the sporangiophores of *Phycomyces blakesleeanus*; John Raper's (1940) discovery of pheromones in the sex life of aquatic fungi by clever manipulation of heterothallic strains of *Achlya ambisexualis* growing in connected microaquaria; and of course the greatest contribution of fungal research to biology: Beadle's and Tatum's (1942) work with auxotrophic mutants of *Neurospora crassa* discovering the one-to-one relationship between genes and proteins.

By the middle of the 20th century the dimorphism of human pathogenic fungi commanded much attention; such fungi can grow either as single yeast-like cells or develop a mycelial morphology (Scherr and Weaver 1953). What made this morphological duality so relevant was the extraordinary fact that pathogenicity was directly correlated with cell shape. Early studies on mycelial-yeast dimorphism were focused on finding the environmental conditions that would elicit one shape or the other. There was the expectation that by identifying the nature of the external effectors of shape clues about the basis of morphogenesis would be revealed. This did not happen; despite many studies and promising leads the nature of the ultimate organizer(s) of morphogenesis remain(s) unrevealed; nevertheless, in the process a great deal was learned about the biology of dimorphic fungi (Gow 1994, Szaniszlo, 2012).

The study of dimorphism took a fresh new direction with the work and insight of Walter Nickerson (1958) who championed investigating questions of fungal morphology by focusing on the cell wall, the structure ultimately responsible for shape. The challenge was to find biochemical pathways linking environmental factors with the chemical composition of the cell wall. Nickerson also advocated elevating investigations on fungal morphology to the realm of morphogenesis. This despite criticism from traditional students of morphogenesis, namely embryologists, who did not think the developmental simplicity of a single-cell organism merited such status. Nevertheless, the concept of morphogenesis was well received by the microbiological community and served to promote and consolidate studies on diverse types of morphological development. Witness the quasi exponential growth in publications that have made reference to fungal morphogenesis during the past six decades (Fig. 1). The new field of fungal morphogenesis grew beyond cell-wall studies and in fact extended to cover all aspects of the

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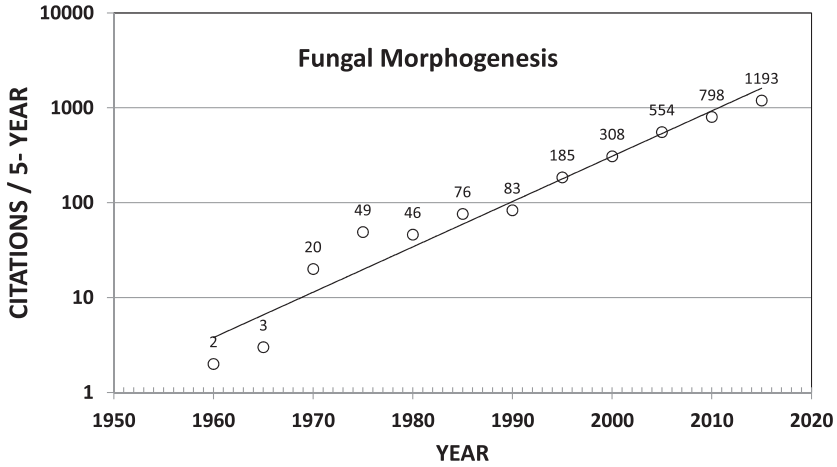

FIG. 1. Frequency of literature citations making specific reference to "fungal morphogenesis". Calculated from Google Scholar in five y periods.

FIG. 2. Gallery of early pioneers in fungal morphogenesis.

structure and function of a fungal cell differentiation (FIG. 2).

During my years in the world of fungal research, I noticed progress moving in waves generated by the arrival of new instruments or techniques or sometimes new ideas. I decided to use these correlations to illustrate significant steps in the evolution of the field of fungal morphogenesis based on my own personal experience.

SHADOW-CAST ELECTRON MICROSCOPY AND X-RAY DIFFRACTION—THE FIBRILLAR NATURE OF THE FUNGAL WALL

The availabilty of electron microscopes after WWII raised great expectations in the biological world, but for many years they failed to deliver the awaited glimpse at the internal structure of fungal cells or any other cells. One area where electron microscopy scored an early success was in revealing the unique microfibrillar nature of the fungal cell wall (and algal walls as well or better). By using shadow-cast techniques to cover the specimens with vaporized metal, Swiss and Dutch researchers (Frey-Wyssling and Muhlethaler 1950, Houwink and Kreger 1953) showed the first images of the cell wall architecture of yeasts and mycelial fungi. Electron microscopy together with X-ray diffractometry made it possible to identify unmistakably the two major microfibrillar polymers of fungal cells: cellulose and chitin and also β-1,3-glucan and chitosan. A survey by Frey (1950) of a large number of fungi proved conclusively that their walls contained either chitin or cellulose and thus helped clarify the taxonomic status of what were then called Phycomycetes. The accumulated knowledge on cell-wall chemistry (Bartnicki-Garcia 1968) together with the discovery by Henry Vogel (1960) of two totally different lysine biosynthetic pathways among fungi was the basis for speculations on the existence of different evolutionary lines in the fungi (Bartnicki-Garcia 1970). Such biochemical information reinforced proposals made by earlier mycologists that Oomycetes and other aquatic fungi had a separate evolutionary origin (Bartnicki-Garcia, 1970). These studies were the forerunner of a revolution in fungal taxonomy that brought about molecular phylogeny (Blackwell et al. 2006, McLaughlin et al. 2009).

CELL RUPTURE AND PAPER CHROMATOGRAPHY—CELL WALL CHEMISTRY

Techniques similar to those used by the bacteriologists to break cells and isolate purified cell walls (Salton and Horne 1951) were adopted and adapted for rupturing fungal cells. The task was much more challenging owing to the toughness of the microfibrillar walls of yeasts and mycelial fungi. Violent agitation with glass microbeads proved most effective, although other methods were tried first. The first reports on the chemistry of purified fungal walls (Aronson and Machlis 1960, Bartnicki-Garcia and Nickerson 1962) were followed by studies on a variety of other fungi (Bartnicki-Garcia 1968). It became clear that chitin and β1,3-glucan were the principal components in most fungi. Paper chromatography proved of tremendous help in identifying monomeric components of fungal cell walls. And X-ray diffractometry was a decisive tool for identifying the structural polymers of the wall (chitin, cellulose, chitosan). Studies on the wall composition at different stages of the life cycle of diverse fungi led to the conclusion that changes in the chemical composition of the cell wall were not the basis for morphological differentiation contrary to initial expectations (Bartnicki-Garcia and Nickerson 1962).

BIOCHEMICAL PATHWAYS—*BLASTOCADIELLA EMERSONII* AND *NEUROSPORA CRASSA*

By the 1950s biochemistry was nearing its apogee; it was the avant garde area of biological research. Biochemical thinking was dominated by pathways connecting linearly a myriad of cell metabolites: glycolysis, TCA cycle, terminal respiration, amino acid biosynthesis and countless other routes. In this environment it seemed reasonable that the biochemistry of morphogenesis could be traced, in direct pathway fashion, from the compound triggering a morphological event to its final product. Although this approach proved to be too simplistic, it is important to recognize some extensive efforts such as those made by Cantino and his group (Cantino et al. 1957) to find a biochemical explanation for how CO_2 brought about the formation of resistant sporangia (RS) in *Blastocadiella emersonii* (i.e. the formation of sporangia with melanized thick walls). In the end he concluded: "Both bicarbonate and ketoglutarate trigger the morphogenetic mechanism … (but) we do not know whether …. they play a direct role in either the synthesis or the degradation of chitin".

Another early setback in the hope of finding direct biochemical explanations for morphogenesis came out from Stuart Brody's (1973) biochemical analysis of morphological mutants of *Neurospora crassa*. The genes affected by some of these mutations encoded enzymes of primary metabolism (e.g. glucose-6-phosphate dehydrogenase in three mutants, *col-2, bal* and *frost*). The immediate consequence of these mutations was the accumulation of glucose-6-P and decrease in NADPH. A straightforward connection between these metabolites and the altered colonial

morphology is difficult to see, especially because the final target that produced the colonial morphology is unknown. A valuable outcome of these studies was the implicit warning about the unexpected biochemical complications created by the pleiotropism unleashed by specific mutations.

PHASE CONTRAST MICROSCOPY—THE SPITZENKÖRPER

By the middle of the 20th century the morphology of fungi and their life cycles were well established. However, knowledge on internal cell structure was limited and confined largely to observations of stained specimens with bright field microscopes. The phase contrast microscope became available after WWII, making it possible to observe internal organelles in unstained living cells. Several investigators took particular advantage of this technique to study nuclear behavior, especially in basidiomycetes where nuclear dynamics has extraordinary features (Girbardt 1955, Bakerspigel 1959). Girbardt's work went beyond nuclear behavior and in the process made one of the most significant advances in hyphal morphogenesis: proof of the existence of the Spitzenkörper (Girbardt 1957). By carefully matching the refractive index of the surrounding medium to that of the cell surface to eliminate the distortion created by the cell wall, he confirmed the existence of the long forgotten Spitzenkörper discovered and so named by Brunswik (1924). Girbardt provided cinematographic evidence of the role of the Spitzenkörper in the apical growth of fungi hyphae placing this organelle at the center of basic research in hyphal morphogenesis.

AUTORADIOGRAPHY—APICAL GRADIENT OF WALL SYNTHESIS

By the late 1960s it became clear that morphological differentiation was not achieved by changes in the chemical composition of the cell walls. The focus then shifted to explore the manner in which the wall is put together. The availability of autoradiographic procedures opened the door to detect with precision the places where a cell wall polymer such as chitin (and chitosan) was deposited by growing fungal cells. The autoradiographs of *Mucor rouxii* young hyphae showed wall synthesis (chitin/chitosan) concentrated at the tip in the form of a sharp gradient emanating from the hyphal pole (Bartnicki-Garcia and Lippman 1969). Similar conclusions were obtained for the polarization of glucan and chitin synthesis in other fungi (Gooday 1971, Katz and Rosenberger 1971). These observations provided a solid base for studies analyzing the nature of polarized cell wall growth in fungal hyphae.

TRANSMISSION ELECTRON MICROSCOPY—A CARNIVAL OF MEMBRANES AND VESICLES

The impact of transmission electron microscopy (TEM) was enormous. For three decades, 1960s–1980s, no biological department with a legitimate claim of studying fungal biology could be found without at least one electron microscope. Ultrastructural research became the subject of numerous doctoral dissertations. Although electron microscopes had been available much earlier, it was not until the late 1950s, when specimen handling was perfected for fixing, embedding and making the required ultrathin sections, that good views of the interior of the fungal cell started appearing in print, for example, *Polystictus versicolor* (Girbardt 1958), *Neurospora crassa* (Shatkin and Tatum 1959), *Uromyces caladii* (Moore and McAlear 1961), *Blastocladiella emersonii* (Cantino et al. 1963), *Rhizoctonia solani* (Bracker and Butler 1963), and continued for many years. These pioneers deserve much credit for developing techniques and setting high standards, accomplishments made more remarkable by the fact that in general fungal cells proved to be most difficult to prepare for TEM. The benefit of TEM reached all aspects of fungal morphogenesis in that it revealed the involvement of internal cell structures in morphogenetic process. Some of the studies provided images of great detail and even beauty illustrating intimate details of the differentiation process in the life cycle of fungi. For instance, the sporulation of *Gilbertella persicaria* (Bracker 1966), the life cycle *Blastocladiella emersonii* (Leslie and Lovett 1968, Lovett 1975), the gametes of *Allomyces macrogynus* (Pommerville and Fuller 1976), the asexual and sexual stages of *Phytophthora* spp. (Hemmes 1983, Hardham 1987) and many others.

Pioneering studies from Charles Bracker and his collaborators (Grove et al. 1968) on *Pythium* were particularly significant in that they disclosed for the first time the continuity of the endomembrane system in the fungal cell. They revealed a progression of membranes that extended from the nuclear membrane to the endoplasmic reticulum to the Golgi-like compartments to secretory vesicles and finally the plasma membrane. Their findings paralleled observations made by George Palade and other animal cell biologists (Dallner et al. 1966) and became the structural foundation of the secretory apparatus central to understanding eukaryotic cell biology. The essence of the secretory apparatus of the fungal cell thus was revealed and the stream of research it created continues unabated.

Two separate articles from the laboratories of Manfred Girbardt and Charles Bracker had a lasting impact by revealing the structural organization of the hyphal tip of a wide variety of fungi hence laying the

cytological basis for hyphal morphogenesis (Girbardt 1969, Grove and Bracker 1970, tribute in Bartnicki-Garcia 2015). Their findings unveiled the cellular secret of apical growth, namely the accumulation of secretory vesicles in the apical region. Most significant was the discovery that the Spitzenkörper was an elaborate aggregate of vesicles of two different sizes, currently named macro- and microvesicles; these findings led to the conclusion that these vesicles were the business end of a secretory apparatus responsible for apical growth. But their classic images missed a key central structure that did not survive fixation, the cytoskeleton. Years later with improvements in fixation and the help of immunolabeling the presence of the microtubular and actin cytoskeletons in the hyphal apex region was revealed (Roberson and Fuller 1988, Bourett and Howard 1991).

Having demonstrated that chitin microfibrils can be made in vitro (Ruiz-Herrera and Bartnicki-Garcia 1974), a search for the identity of the particles carrying chitin synthase in the cell was initiated. It turned out that they were microvesicles such as those seen under TEM (Bracker et al. 1976). These microvesicles named chitosomes first were isolated from *Mucor rouxii* by density-gradient centrifugation and characterized by negative staining TEM (Bracker et al. 1976). Isolated chitosomes retain full enzymatic activity including zymogenicity. Upon incubation with substrate (UDP-GlcNAc) they produced chitin microfibrils in vitro. The unique physical properties (size and specific gravity) of chitosomes allowed their purification. The same unfortunately has not been so with other larger vesicles that pose a great challenge in separating them from the mix of membranes resulting from cell homogenization.

PERSONAL COMPUTER—MATHEMATICAL MODELS

Beginning with the cartographic approach of Reinhardt (1892) mentioned above, a number of attempts were made to describe in mathematical terms the pattern of surface growth that could generate a nearly cylindrical shape from a quasi hemiellipsoidal tip (da Riva Ricci and Kendrick 1972, Bartnicki-Garcia 1973, Trinci and Saunders 1977, Prosser and Trinci 1979, Koch 1982). They, however, were basically geometric exercises that formulated equations without involving actual features of the growth process. In the 1980s we made an attempt to develop a more realistic mathematical model of hyphal growth based on what are clearly the main subcellular structures involved in cell wall growth in fungi: secretory vesicles. The model quantitated an earlier scheme of fungal wall growth based on vesicles (Bartnicki-Garcia 1973). With the help of two enthusiastic mathematicians, Gerhard Gierz and Fred Hergert, we agreed that the solution was not in concocting abstract equations but in taking advantage of a new tool: the personal computer. A program was written to imitate the growth of a cell based on the idea that the growth of a cell is generated by the collective discharge of vesicles. Pixels became the vesicles emanating from an imaginary vesicle source. The simulations led to an Eureka moment: the realization that a sustained linear displacement of the source of vesicles (VSC) releasing them in all directions generates cylindroid hypha-like shapes with the peculiar tapered tip (Bartnicki-Garcia et al. 1989). Notwithstanding the extreme reductionism involved, there was a striking functional similarity between the VSC generating the profile of a tube and the behavior of a Spitzenkörper in a real growing hypha. The entire secretory process could be described by a simple equation $y = xcot(Vx/N)$ embodying the interplay between the number of vesicles (N) and the rate of advance (V) of the VSC. A plot of this equation generates the hyphoid, the ideal shape of a hypha. The vesicle supply center concept (VSC) offered a credible explanation for the basic role of the Spitzenkörper in apical growth namely a distribution center for wall-building vesicles. The VSC model fits Frank Harold's (2005) assessment of productive reductionism: "if the reductionist agenda is to be productively applied to living systems, computation must begin from physiological processes rather than molecules or genes". The VSC is an abstract concept, elucidating details of how the Spitzenkörper manages to receive, process and release vesicles remains a formidable task that is being investigated in laboratories around the world.

MOLECULAR BIOLOGY AND PCR TECHNOLOGY

Hydrpydrophobins, γ-tubulin and much more.—In the 1960s the concepts and techniques of molecular biology spread quickly to all quarters of biology including mycology. The central dogma, the chain of information going from DNA to RNA to proteins, became rapidly the holy grail of researchers hoping to find the macromolecular connection between gene expression and specific morphogenetic processes. By the 1990s PCR machines became common place making molecular genetics practical and popular. DNA amplification became a method of choice to tackle questions related to morphogenesis. Advances were made in a variety of morphogenetic topics on widely diverse fungi.

One of the most elegant early studies on fungal molecular biology was done by James Lovett (1968) with a zoosporic fungus, the chytridiomycete *Blastocladiella emersonii*. By astute use of radioisotopes and specific inhibitors of the synthesis of DNA, RNA and proteins he showed how the fungus programmed and

preprogrammed macromolecular synthesis for the subsequent steps in the course of differentiation from zoospores to cysts to sporangia.

Some noteworthy contributions of this era deserve credit: Ronald Morris and Xin Xiang for understanding the role of microtubules and their motors in nuclear migration (Xiang et al. 1995a, b); William Timberlake and collaborators for discovering transcription regulators in the molecular cascade involved in *Aspergillus* conidiation (Adams et al. 1988). Jay Dunlap and Jennifer Loros for identifying the genes regulating the circadian rhythm of *Neurospora crassa* (Loros et al. 1989, Dunlap and Loros 2004;). Regine Kahmann and Lorna Casselton and their associates for deciphering the molecular intricacies of the mating types of *Ustilago maydis*, *Schizophyllum commu*ne and other Basidiomycetes (Kahmann and Bölker 1994, Feldbrügge et al. 2004, Casselton and Olesnicky 1998). Berl Oakley's discovery of γ-tubulin in *Aspergillus nidulans* and its role in microtubule assembly is another outstanding contribution of fungal research with universal repercussion in biology (Oakley et al. 1990). An extensive study by Joseph Wessels and his associates (Wessels et al. 1991) on the molecular changes accompanying sexual development of the Basidiomycete *Schyzophyllum commune* revealed the presence of genes coding for proteins unique to fungi, the hydrophobins. These extraordinary proteins endowed with hydrophilic and hydrophobic properties coat the outer layer of the cell wall and are important in the formation of aerial hyphae and fruit body morphogenesis.

Facing the avalanche of studies on the molecular biology of fungi, Harold (2005) challenged us to ponder whether molecular genetics could solve the riddle of morphogenesis. He formulated several critical questions: "Is cellular architecture explicitly spelled out by genes, and if so how? How is spatial organization passed from one generation to the next? How do molecules find the correct location in the cell space? What is the origin of large-scale order … of eukaryotic cells? How do multitudes of molecules reproducibly come together into cellular forms?" They represent enticing marching orders for present and future fungal biologists.

VIDEO MICROSCOPY—THE ERA OF LIVE CELL IMAGING

Video microscopy liberated researchers from the tedium of observation through the microscope eyepieces and much more. Video microscopy allowed the electronic manipulation of the image enhancing contrast and making it practical to record digitized images of fungal behavior for future analysis. Quantitative video microscopy of images analyzed second by second led to the discovery of the pulsatile nature of hyphal growth (Lopez-Franco et al. 1994). The novelty of the procedures used to detect pulses elicited unwarranted skepticism from Jackson (2001), but her arguments were refuted by Money (2001). Sampson et al. (2003) dismissed summarily the existence of pulses in fungal hyphae because they showed no strict periodicity and preferred to call them "random fluctuations". Regardless of this strict definition of pulsation the fact is that hyphae pulsate; the pulses observed are not perfect but pertain to a living cell with all its inherent variabilities. Video microscopy with laser beam trapping let Charles Bracker and Rosamaria Lopez-Franco manipulate the Spitzenkörper thus providing convincing proof that the Spitzenkörper dictates the directionality of hyphal growth (Bracker et al. 1997); this finding runs contrary to the common belief that the polarized growth of a hypha may be guided by surface markers.

CONFOCAL MICROSCOPY AND MOLECULAR GENETICS—ILLUMINATING THE SECRETS OF THE HYPHAL TIP

The art and science of covalently attaching fluorescent tags, mainly GFP or its variants, on a selected protein, followed by examination of the living tagged cell under the confocal or the epifluorescence microscope, has become one of the most powerful tools in today's arsenal of fungal biology. This methodological duo has been particularly useful in elucidating the place and role of components of the apical growth apparatus of hyphae.

A major revelation was the finding that chitin synthases (CHS) located preferentially in the hyphal tips of several fungal species: *Aspergillus nidulans* (Takeshita et al. 2005), *Ustilago maydis* (Weber et al. 2006); *Neurospora crassa* (Riquelme et al. 2007) in harmony with the previous evidence that found chitin synthesis sharply concentrated in the hyphal tips (Bartnicki-Garcia and Lippman 1969, Gooday 1971). The studies with *N. crassa* showed with greater precision that several CHS were located in the core of the Spitzenkörper where microvesicles congregate, hence the obvious conclusion that the Spitzenkörper core was a collecting site for chitosomes. These in vivo findings meshed neatly with previous in vitro studies that identified chitosomes as the microvesicular conveyors of CHS in fungal cells (Bracker et al.1976). Another major finding pertained to the enzyme complex responsible for the synthesis of β-1,3-glucan, which was located separately in the macrovesicles that comprise the Spitzenkörper outer zone (Verdin et al. 2009, Sanchez-León and Riquelme 2015). The reason behind this intriguing biochemical stratification of the Spitzenkörper, with chitin-making microvesicles in the inner zone and glucan-making macrovesicles in the outer zone, is yet to be elucidated. Recent

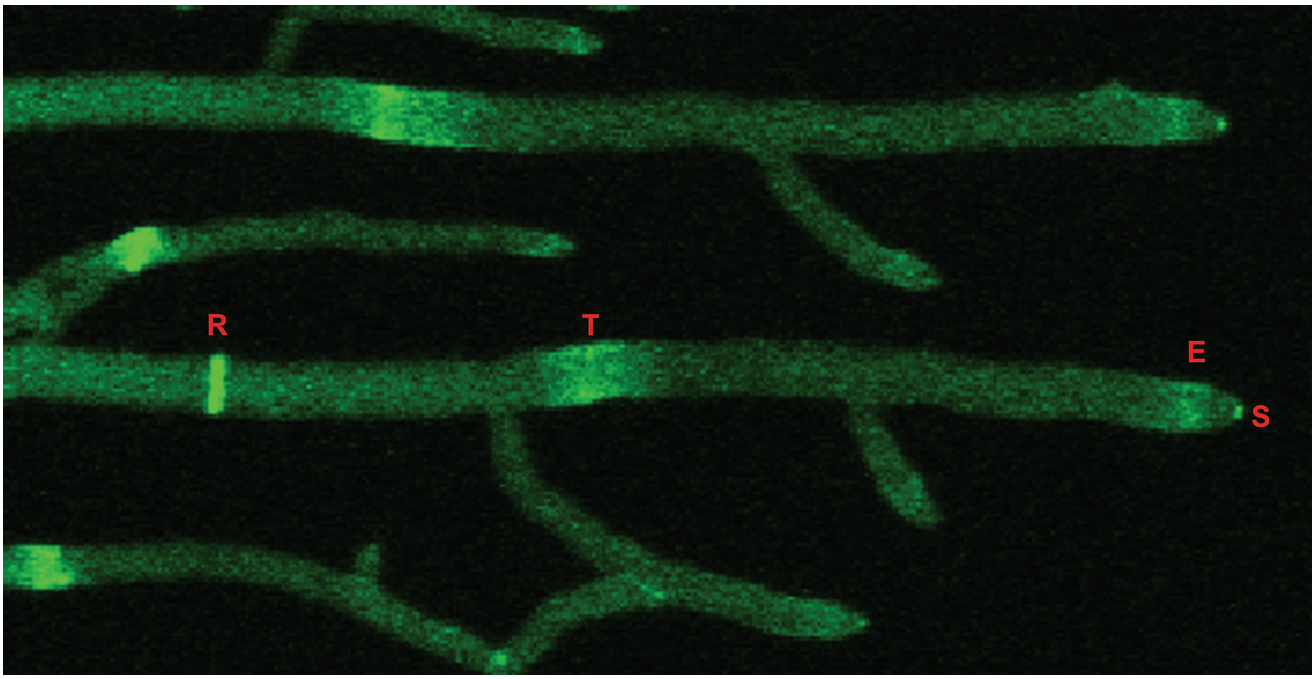

FIG. 3. Actin in action. Growing hyphae of *Neurospora crassa* tagged with Lifeact-GFP showing the main sites of actin activity namely: S. The core of the Spitzenkörper. E. The endocytic subapical collar. T. A septal actomyosin tangle. R. The actomysin ring of a fully formed septum (Delgado-Alvarez et al. 2014). (Image from a video courtesy of Rosa Mouriño-Perez).

studies from Riquelme's lab found that these two kinds of vesicles associate with different Rab GTPases, an indication that they arrive at the Spitzenkörper by separate routes (Sanchez-León et al. 2015).

The Spitzenkörper is not alone in the apex; a large number of other proteins have been located in the apical region. These proteins are members of two groups, the polarisome and the exocyst (Harris et al. 2005, Riquelme and Sanchez-León 2014). The former are proteins that mediate the nucleation of actin microfilaments and are involved in regulating vesicle flow (Stephenson et al. 2014). Exocyst proteins serve to tether vesicles to exocytosis sites. While the mere presence of these proteins signals their participation in hyphal morphogenesis, their essentiality is not always clear. For instance, deletion of the master regulator cdc42 causes severe inhibition of growth but it did not eliminate polarized apical growth in the Δ-mutant of *N. crassa* (Araujo-Palomares et al. 2007).Molecular tagging plus confocal microscopy also has been a great asset in revealing details of the operation of the fungal cytoskeleton. Michael Freitag's construction of a GFP expression plasmid became a versatile tool first used to demonstrate the relationship of microtubules and nuclei in *Neurospora crassa* (Freitag et al. 2004). Lifeact-GFP tagging permitted mapping the presence and dynamics of actin in the living hyphal cell. Some of the key roles played by actin can be displayed in spectacular fashion by watching growing hyphae tagged with lifeact (FIG. 3): A sharp fluorescent signal in the core of the Spitzenkörper announces its central role in apical growth, a dynamic collection of patches in the subapical collar role shows endocytosis in action and unique actin structures such as the septal actomyosin tangle precede the development of a septum (Delgado-Alvarez et al. 2014). While the actin cytoskeleton is involved in the traffic of vesicles from the Spitzenkörper to the cell surface, the microtubular cytoskeleton remains a major route for secretory vesicles to reach the apical region (McDaniel and Roberson 2000, Horio and Oakley 2005, Takeshita and Fischer 2011). Another example of the benefit of combining molecular markers and confocal microscopy was the discovery of the extraordinary cross talk that takes place during hyphal anastomosis (Read et al. 2012).

CONCLUSION—HYPHAL MORPHOGENESIS—A STILL MYSTERIOUS VESICLE DANCE

For the past 60+ years we have seen the field of fungal morphogenesis evolve exponentially. The avalanche of information catalyzed by the availability of new technology showed us what is inside the fungal cell, plus a variable degree of understanding of how internal structures function.

Hyphal morphogenesis received a great deal of attention, understandably so, because the polarized

construction of tubular cells is the hallmark and raison d'etre of the Fungi. Add to it their obvious but deceiving simplicity inviting experimentation. Do we understand hyphal morphogenesis? Not yet. Missing is the nature of the master orchestrator, if there is indeed a single one, guiding the organized vesicle dance that is polarized exocytosis; a vesicle dance whose final discharge pattern generates the predicted gradient of wall growth that would produce a tubular cell. A germinating spore, its wall machinery illuminated by tritium tracing, is a good subject to ponder on the onset of polarity (Bartnicki-Garcia and Lippman 1977) and wonder about the nature of the quiet switch that, without disturbing metabolism, turns exocytosis from an isotropic dance to a polarized one.

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