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Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems

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Abstract: The ability of rapidly growing hyphae to generate new polarity axes that result in the formation of a branch represents one of the most important yet least understood aspects of fungal cell biology. Branching is central to the development of mycelial colonies and also appears to play a key role in fungal interactions with other organisms. This review presents a description of the two major patterns of hyphal branching, apical and lateral, and highlights the roles of internal and external factors in the induction of branch formation. In addition, potential mechanisms underlying branch site selection are outlined, and the possible roles of multiple signaling pathways (i.e., G protein α , Cdc42, NDR kinases) and subcellular structures (i.e., the Spitzenkorper, septins) are discussed. Finally, other forms of branching in the plant and animal kingdoms are briefly summarized and compared to hyphal branching.

Key words: apical branch, hyphal morphogenesis, lateral branch, polarity establishment

INTRODUCTION

The success of fungi in colonizing terrestrial ecosystems can be largely attributed to their ability to form hyphae and mycelia (Rayner et al 1995). Hyphae are highly polarized cylinders that usually grow by apical extension at rates that can approach $\geq 1 \mu\text{m/s}$ (Seiler and Plamann 2003). Fungal hyphae are typically composed of multiple cells demarcated by septa (Carlile 1995). This modular pattern of organization contributes to the differentiation of hyphae; apical cells (or hyphal-tip cells) are generally engaged in nutrient acquisition and sensing of the local environment, whereas sub-apical cells generate new hyphae by lateral branching. The resulting network of hyphae is known as a mycelium. Hyphal branching appears to serve two general purposes. First, it increases the surface area of the colony, which presumably enhances nutrient assimilation. Second, branches mediate hyphal fusion events that appear to be important for

exchange of nutrients and signals between different hyphae in the same colony. Nevertheless, little is known about the molecular basis of hyphal branching. Although the molecular processes involved in polarized hyphal growth would obviously be needed for the formation and growth of a branch, the recent identification of mutants with branching-specific phenotypes suggests that branch formation does not involve a simple recapitulation of the polarity establishment mechanisms that underlie polarization of germinating spores.

The characteristic pattern of mycelial organization implies that individual fungal hyphae exhibit a phenomenon known as apical dominance, whereby the growing tip is dominant and suppresses the formation of lateral branches in its vicinity (Schmid and Harold 1988, Semighini and Harris 2008). This phenomenon likely reflects the exclusive targeting of exocytic vesicles laden with components required for cell surface expansion and cell wall deposition to the hyphal tip at the expense of potential branching sites. These sites presumably become active only when they are a sufficient distance from the hyphal tip. It seems intuitive that the absence of apical dominance would result in a chaotic growth pattern that compromises colony development, and indeed, recent genetic analysis supports this view (Semighini and Harris 2008). Accordingly, the existence of apical dominance suggests that hyphal branching is subject to temporal and spatial regulatory mechanisms that ensure normal patterns of mycelial organization.

The primary objective of this review is to draw attention to the process of hyphal branching as an essential feature underlying the development of fungal colonies. The importance of hyphal branching cannot be understated. For example, it plays a pivotal role in both beneficial and detrimental interactions between fungi and plants. In addition, the control of hyphal branching is a significant issue in the fermentation industry. Accordingly, a better understanding of hyphal branching and its regulation is a desirable goal. The first part of the review will provide an overview of the phenomenon of hyphal branching and will include a description of known branching patterns as well as a discussion of examples where branching is modulated in response to external factors. The second part of the review will emphasize possible mechanisms that determine where and how branches form. This will encompass older physiolog-

ical studies that have been extensively summarized in previous reviews (Trinci 1978), as well as newer results derived from recent genetic analyses. Finally, other branching systems, such as developing neurons, will be described briefly with the intent of drawing broad analogies that might be instructive for understanding the process of hyphal branching.

BRANCHING PATTERNS

Apical branching.—The emergence of a branch from the hyphal tip is referred to as apical branching. This pattern of branching has been observed in a large number of fungi (Trinci 1978). In many of these fungi, apical branching presumably occurs in response to the abnormal accumulation of exocytic vesicles at the hyphal tip. This could conceivably be triggered by perturbations that slow extension of hyphal tips without interrupting the flow of exocytic vesicles through the cytoplasm. Because the supply of vesicles exceeds their capacity to be incorporated into the existing tip, they accumulate leading to the formation of a new tip (Katz et al 1972, Trinci 1974) (FIGS. 1, 2A). Although numerous mutations that cause increased apical branching (also referred to as dichotomous branching or tip splitting) have been described in fungi such as *A. nidulans*, *A. niger* and *N. crassa* (Trinci and Morris 1979, Reynaga-Pena and Bartnicki-Garcia 1997, Gavric and Griffiths 2003, Virag and Griffiths 2004, Virag and Harris 2006), the fact that it occurs in wildtype isolates suggests that it is not merely an abnormal pattern (Riquelme and Bartnicki-Garcia 2004). Furthermore, there is limited evidence that apical branching shares common control mechanisms with the more prevalent branching pattern, lateral branching (Watters and Griffith 2001). Instead, it seems likely that apical branching is a general response that enables continued growth under conditions that compromise organization of hyphal tips and thereby disrupts apical dominance.

There are fungi for which apical branching appears to be a programmed feature associated with rapid hyphal extension. A well-characterized example of this behavior is exhibited by *Ashbya gossypii*, a member of the Saccharomycotina. In *A. gossypii*, hyphae initially undergo lateral branching as they steadily increase their extension rates from 5 $\mu\text{m}/\text{h}$ to a maximum of 170 $\mu\text{m}/\text{h}$ (Philippsen et al 2005). Once they reach this rate, at a point referred to as hyphal maturation, they switch to an apical branching pattern. The advantage of this switch is unclear, but it might reflect the inherent inability of a single *A. gossypii* tip to accommodate the massive influx of vesicles needed to sustain maximal rates of hyphal extension. The phylogenetic relatedness of *A. gossypii*

FIG. 1. A schematic drawing of a fungal hypha that shows both apical and lateral branching patterns. The filled black oval represents the spore. Tick marks on the primary hyphae represent septa.

to the model yeast *Saccharomyces cerevisiae* has facilitated the identification of genes involved in hyphal morphogenesis, including examples that are specifically required for apical branching (i.e. Cla4, Pxl1, Spa2) (Ayad-Durieux et al 2000, Knechtle et al 2003, Knechtle et al 2008).

Lateral branching. The predominant branching pattern exhibited by fungal hyphae is lateral branching, whereby new branches emerge from sites distal to the hyphal tip (FIG. 1, 2A–C). Several features distinguish the formation of lateral branches from apical branching (Riquelme and Bartnicki-Garcia 2004). In particular, unlike apical branching, the formation of a lateral branch has no apparent impact on the extension rate of a growing hypha or the shape of its tip. In addition, lateral branching appears to be associated with the *de novo* formation of a Spitzenkörper near the incipient branch site, whereas apical branching is triggered by the temporary loss of the Spitzenkörper at the tip. Because these observations were made using rapidly growing *N. crassa* hyphae, they may not apply to all fungal hyphae. Nevertheless, they are consistent with the idea that the hyphal tip harbors factors that maintain its integrity and suppress branching (i.e., apical dominance; Schmid and Harold 1988, Semighini and Harris 2008). Lateral branching would only occur when a potential site is far enough removed from the tip so as to escape the effects of these factors. Accordingly, the nature of these factors and their mode-of-action is of great interest (see below).

There appears to be two broad patterns of lateral branching; branches associated with septa, and random branching. In the former pattern, new branches emerge adjacent to septa (FIG. 2C), and it seems likely that some component(s) of the septum

FIG. 2. Branching patterns in fungal mycelia. A. Branched hyphae from the leading edge of a growing *Neurospora crassa* (strain FGSC9716) colony. Asterisks mark examples of apical branching. B. Segment of an *Aspergillus nidulans* (strain FGSC28) hypha stained with Calcofluor White (to show septa) and Hoechst 33258 (to show nuclei). Three lateral branches are shown; 1 and 2 emerge from the middle of their respective compartments, whereas 3 appears to be associated with a septum. C. Segment of a *Galactomyces candidum* (strain NRRL Y17569) hypha stained with Calcofluor. Both lateral branches are associated with septa. D. Hyphal segment from an *A. nidulans sepH1* mutant (strain AKS71) stained with Calcofluor. Note the presence of both apical and lateral branches. Bars, 30 μm (A), 3 μm (B–D).

provides a spatial cue that specifies the position of the branch. Several fungi exhibit this pattern (Trinci 1978), including members of the Saccharomycotina (*A. gossypii*, *Geotrichum candidum*), as well as zygomycetes (*Basidiobolus ranarum*) and basidiomycetes (*Coprinus* species). Note that in *A. gossypii*, lateral branching predominates during the early stages of growth that precede hyphal maturation and the switch to apical branching (Philippsen et al 2005). In most cases of lateral branching, the branch emerges just behind the septum, which would be expected if the septum were serving as a barrier that impeded the tip-bound flow of exocytic vesicles and thus led to their local accumulation. However, the analysis of the *A. gossypii bud3* mutant suggests that this interpretation may be too simple. In this mutant, delocalization of actin rings at septation sites results in the accumulation of aberrant chitin aggregates instead of normal septa (Wendland 2003). Nevertheless, branches still emerge from these sites. It is tempting to speculate that a component involved in an early step in septum formation (i.e. the septins; Gladfelter et al 2001, Gladfelter 2006, Pan et al 2007) may provide a spatial cue for branch formation.

The random pattern of lateral branching is observed primarily in those ascomycetes that belong to the Pezizomycotina and is characterized by the apparent absence of an association between septation and branching (FIG. 2B). Quantitative analysis reveals that branches tend to emergence from the center of sub-apical cells in *N. crassa*, whereas there might be a slight bias toward the apical septum in *A. nidulans* (Trinci 1978, Walther and Wendland 2003). A common feature of these fungi is the formation of incomplete septa that might be less effective barriers to vesicle flow than the complete or dolipore septa formed by those fungi that branch in association with septa (Trinci 1978). How, then, is the branch site determined in fungi such as *N. crassa* or *A. nidulans*? It could simply be the stochastic accumulation of vesicles at a cortical site that triggers branch formation (i.e. Katz et al 1972, Trinci 1974). On the other hand, there is limited evidence that localized calcium or ROS spikes may specify potential branch sites (Grinsberg and Heath 1997, Semighini and Harris 2008). Finally, it seems likely that localized nuclear division could also play a critical role in determining where and when a branch forms.

At this point, it should be noted that septum formation is not a *de facto* requirement for the formation of lateral branches. Many zygomycetes, such as *Rhizopus* and *Mucor* species, undergo lateral branching despite the formation of aseptate hyphae (Trinci 1978). In addition, *A. nidulans* and *N. crassa* mutants defective in septum formation remain

capable of branching (e.g., Morris 1975, Harris et al 1994, Rasmussen and Glass 2005, FIG. 2D). Nevertheless, the presence of septa might play a key role in regulating the timing of lateral branch formation.

Coordination of branching with the cell cycle. For at least some filamentous fungi, the formation of lateral branches from a sub-apical cell is coordinated with the cell cycle. For example, Fiddy and Trinci (1976) reported that in *A. nidulans*, new sub-apical cells enter a period of cell-cycle arrest before a new branch emerges. A similar phenomenon has been observed in *C. albicans* hyphae (Gow and Gooday 1982). Subsequent studies in *C. albicans* suggest that a cell size-control mechanism prevents branch formation by restraining entry into the G1 phase of the cell cycle until sub-apical cells have accumulated sufficient levels of cytoplasmic volume (Barelle et al 2006). In *A. nidulans*, the maintenance of an appropriate ratio of cytoplasmic volume per nucleus appears to be an important determinant that promotes branch formation adjacent to nuclei that are actively dividing (Dynesen and Nielsen 2003). Furthermore, in *A. gossypii* hyphae, mitosis occurs more frequently at branching sites than would be expected if the two processes were not coordinated (Helfer and Gladfelter 2006).

Although these results collectively demonstrate the coordination between branching and the cell cycle, the underlying mechanism remains obscure. However, the morphogenetic checkpoint of *S. cerevisiae* may provide a useful paradigm. This checkpoint blocks cell-cycle progression until a bud is available to receive a newly divided nucleus (Keaton and Lew 2006). Key features of this checkpoint include the septin-dependent recruitment of cell-cycle regulators such as Wee1 to the mother-bud neck. The localization of septins to incipient branch sites in both *A. gossypii* and *A. nidulans* (Westfall and Momany 2002, Helfer and Gladfelter 2006) suggests that they might function in the same way to enforce local cell-cycle arrest until a new branch has formed.

REGULATION OF BRANCHING BY EXTERNAL FACTORS

Branch formation is clearly an intrinsic feature of fungal hyphae that presumably underlies the ability of filamentous fungi to form a mycelium. However, it is well established that branch formation can also be regulated by external factors. This is particularly evident during fungal interactions with plants; examples where branching is induced have been amply documented, whereas in other cases it seems likely that branching is suppressed. The localized induction of branching may also play a role in promoting intra-hyphal fusion events during the development of a mycelium.

The establishment of mycorrhizal associations between filamentous fungi and land plants involves a series of interactions between growing hyphae and plant root systems (Paszkowski 2006). Arbuscular mycorrhizal (AM) fungi belonging to the genera *Gigaspora* and *Glomus* are members of the Glomeromycota that have been used to study the early stages of host recognition that precede the formation of appressoria (Giovanetti et al 1993, Giovanetti et al 1994, Akiyama et al 2005). In the absence of a prospective host, spores from these fungi germinate, but the resulting hyphae grow slowly and exhibit apical dominance. This pattern changes dramatically, however, in the presence of root exudates derived from the host. These exudates contain factors that stimulate spore germination and, most importantly, abolish apical dominance and trigger profuse hyphal branching. Analysis of exudates from the legume *Lotus japonicus* identified the “branching factor” as a strigolactone (Akiyama et al 2005), a class of plant hormones derived from carotenoids that inhibit shoot branching and also mediate communication with parasitic weeds (e.g. Umehara et al 2008). The mechanism by which strigolactones promote hyphal branching and the extent to which they act on other fungi are important areas of further investigation.

Like mycorrhizae, the synthesis of lichens involves a series of early interactions between the fungal partner (i.e. the mycobiont) and the algal or cyanobacterial partner (i.e. the photobiont). Though not as well characterized as the interactions between mycorrhizal partners, detailed microscopic analysis shows that the lichen mycobiont loses apical dominance and undergoes extensive hyphal branching during its initial interaction with the photobiont (Ahmadjian and Jacobs 1981, summarized in Ahmadjian 1993). Several candidates have been suggested for the photobiont-derived signal that elicits this response, including plant hormones (IAA, kinetin) and the sugar alcohol, ribitol (Ahmadjian 1993). However, it seems likely that, as with mycorrhizae, a small molecule such as strigolactones will emerge as the lichen “branching factor.” Notably, the ability of the photobiont to produce this factor appears to require light (Ahmadjian 1993).

Hyphal fusion is thought to play a key role in the development of fungal mycelia by facilitating the exchange of nutrients and signals between neighboring hyphae (reviewed in Rasmussen et al 2004). Live imaging analysis has provided evidence for chemotropic interactions between fusing partners prior to contact. In particular, Hickey et al (2002) show that a *N. crassa* hyphal tip can induce the formation of a new lateral branch from an adjacent hypha. This likely involves the secretion of a diffusible factor that

triggers branching, perhaps by promoting the formation of a new Spitzenkörper at the incipient branch site (Glass et al 2004). Furthermore, recent evidence suggests that a MAP kinase-mediated signaling pathway might mediate the localized morphogenetic response to this factor (Glass et al 2004, Pandey et al 2004).

Whereas the induction of hyphal branching in response to plant or fungal signals has been documented, there are no comparable studies showing that external signals can actively suppress branching. Nevertheless, this would seem to be a reasonable possibility. When the plant pathogen *Claviceps purpurea* infects rye ovarian tissue, it forms hyphae that exhibit extreme apical dominance until they reach the vascular tissue, whereupon they undergo extensive branching (Rolke and Tudzynski 2008). The related endophytic fungus *Epichloe festucae* forms long, unbranched hyphae in ryegrass leaves before switching to a branched pattern of morphogenesis during the formation of stroma (Scott 2001). Remarkably, Christensen et al (2008) recently reported the existence of stable zones of branched and unbranched growth immediately adjacent to each other in a single ryegrass leaf blade, thereby highlighting the precise nature of this morphogenetic switch. Finally, following conidial germination on a permissive surface, the grass pathogen *Magnaporthe oryzae* forms hyphae that do not branch prior to the formation of appressoria (Caracul-Rios and Talbot 2007). Once inside the plant, however, *M. oryzae* hyphae branch profusely (Kankanala et al 2007). In each of these cases, signals derived from the host presumably prevent branching until hyphae reach the appropriate location in the plant. The identification of these hypothetical signals should be an interesting subject for future research.

HOW ARE BRANCH SITES SELECTED?

The analysis of lateral branching patterns suggests two possible models that could explain how filamentous fungi select branch sites: the “septum as a barrier” model and the spontaneous polarization model. Because apical branching is not usually associated with septa, it is more likely to be directed by the latter model.

The “septum as a barrier” model for branch site selection is based on the idea that complete septa impede the tip-ward flow of exocytic vesicles, thus leading to their accumulation just behind newly formed septa (Trinci 1978). According to this model, the subsequent fusion of these vesicles with the hyphal wall would generate a new tip that grows into a branch. However, the proximity of new branches to

septa could also be explained if they share a common molecular component. For example, the septins are likely to be involved in both septation and branching (Westfall and Momany 2002, Gladfelter 2006), and would thus be ideally positioned to direct the formation of a branch next to a newly formed septum. Indeed, this may explain why *A. gossypii* *bud3* mutants form branches adjacent to abnormal septa that would not be expected to block vesicle transport (Wendland 2003).

Spontaneous polarization has been described and modeled in *S. cerevisiae*, where it is proposed to provide a mechanism for polarity establishment in the absence of any known spatial marker (Wedlich-Soldner et al 2003, Altschuler et al 2008). It is based on the premise that local levels of polarity determinants, such as the monomeric GTPase Cdc42, fluctuate in a random manner until they exceed a given threshold at a specific site. This triggers a series of positive and negative feedback loops that reinforce the local polarization signal and thereby enable the formation of a stable polarity axis. Key elements implicated in these feedback loops include actin filaments as well as the coupling of localized vesicle exocytosis to endocytosis from flanking sites (Irazoqui et al 2005, Marco et al 2007). A similar mechanism could reasonably be invoked to explain how branch sites are selected in sub-apical hyphal cells. In this case, the nature of the key branching determinant(s) that accumulates to threshold levels remains an enigma. Obvious candidates include monomeric GTPases such as Cdc42 or Rac1 (see below), as well as molecules such as calcium or reactive oxygen species (ROS). Notably, there is evidence that localized accumulation of calcium or ROS may promote the formation of new tips at sub-apical sites (Jackson and Heath 1993, Grinberg and Heath 1997, Semighini and Harris 2008). Whether this occurs in coordination with monomeric GTPases or other signaling pathways is an important subject that merits further investigation. Finally, the spontaneous polarization model can also account for the effects of external factors on branch formation. In *S. cerevisiae*, mating pheromones act via receptors to bias the choice of a polarity axis so that the cell extends toward the pheromone source (Madhani 2007). Branching factors (i.e. strigolactones) may act in the same way to bias the selection of branch sites.

HOW ARE BRANCHES FORMED?

A coherent picture has yet to emerge of the molecular mechanisms that underlie the formation of a hyphal branch. However, the use of several complementary approaches has begun to reveal the proteins and

processes that are involved in branch formation. These approaches include the identification and characterization of branching mutants, the reverse genetic analysis of genes implicated in hyphal morphogenesis, the study of calcium gradients, and the microscopic analysis of structures such as the cytoskeleton and Spitzenkorper. Results acquired so far suggest that the process of forming a hyphal branch can be conveniently broken down into a series of steps that follow the initial selection of the branch site (Seiler and Plamann 2003). The first step ("recruitment") corresponds to the period during which the morphogenetic machinery (i.e. the components of the cytoskeleton and vesicle trafficking systems required for localized cell surface expansion and cell wall deposition) is recruited to the incipient branch site. The second step ("polarization") refers to the period during which the morphogenetic machinery functions to generate a stable polarity axis that directs emergence of the new branch. The third and final step ("maturation") represents the period during which the new hyphal tip matures and attains its maximal extension rate. Although these steps largely mimic those thought to underlie the emergence of a germ tube from a germinating spore, limited genetic evidence hints at the existence of functions that are specific to branch formation.

Recruitment. Genetic analyses have identified multiple functions that appear to be required for the recruitment of the morphogenetic machinery to incipient branch sites. In *A. nidulans*, both heterotrimeric (i.e. FadA) and monomeric (i.e. Cdc42) GTPases have been implicated in the regulation of branching. Mutations that affect these GTPases lead to the formation of hyphae that are unusually straight and devoid of lateral branches (Virag et al 2007, S.D. Harris unpubl results). Similar phenotypes have been observed when heterotrimeric GTPase function is perturbed in other filamentous fungi (i.e. *Cochliobolus heterostrophus*, *Fusarium oxysporum*, *Alternaria alternata*; Ganem et al 2004, Delgado-Jarana et al 2005, Yamagishi et al 2006), thereby suggesting that G protein alpha may have a universally important role in recruiting the morphogenetic machinery to branch sites. On the other hand, the role of monomeric GTPases such as Cdc42, Rac1 and Ras in hyphal branching remains uncertain, though they are attractive candidates as downstream effectors of heterotrimeric GTPases.

Other functions implicated in the recruitment step include formins and septins, which both have key roles in multiple aspects of hyphal morphogenesis (Gladfelter 2006). Mutations affecting the *A. nidulans* formin SepA abolish lateral branching and trigger increased apical branching (Trinci and Morris 1979,

Harris et al 1997). Because formins nucleate the polymerization of actin filaments, these results suggest that these filaments are required for recruitment of the morphogenetic machinery to branch sites (i.e. perhaps as components of feedback loops that support spontaneous polarization). In *A. gossypii* and *A. nidulans*, septins localize to branch sites, where they could conceivably function as scaffolds for the assembly of multiple morphogenetic complexes (Gladfelter et al 2001). Notably, both formins and septins are known downstream effectors of Cdc42 (Park and Bi 2007), which could explain how they themselves are recruited to incipient branch sites.

Polarization and maturation. A large-scale screen for mutations affecting hyphal morphogenesis in *N. crassa* identified several functions required for the formation of a stable polarity axis at branch sites (Seiler and Plamann 2003). Mutations affecting these functions typically abort branch formation, leading to the formation of small needle-like projections. Functions implicated in branch polarization include NDR kinases, the Rho1 GTPase module and glucan synthase. Studies in other fungi (i.e. *A. nidulans*, *C. purpurea*) have also implicated NDR kinases and Rho1 in the control of hyphal branching (Guest and Momany 2004, Scheffer et al 2005, Johns et al 2006). Whether these functions are downstream targets of G protein α or Cdc42 remains an important question for future investigation.

Detailed microscopic analysis of living hyphae has yielded additional insight into the polarization of branch sites. For example, analysis of Spitzenkörper ontogeny in *N. crassa* suggests that the new polarity axis is stabilized much sooner at branch sites than it is during germ tube emergence. Young germ tubes exhibit erratic growth until they reach a length of $\sim 150 \mu\text{m}$, at which time a Spitzenkörper becomes evident and growth becomes directional (Araujo-Palamares et al 2007). By contrast, organization of a Spitzenkörper is apparent even at the earliest sign of branch emergence (i.e. deformation of the hyphal wall at the incipient branch site, Riquelme and Bartnicki-Garcia 2004), thus implying that a stable polarity axis already exists by this time.

The subsequent maturation of new branches appears to be associated with microtubule function. Cytoplasmic microtubules first become associated with the branch site at the same time the Spitzenkörper appears (Mourino-Perez et al 2006). Further microtubule organization at the branch site appears to reflect both the pulling of existing microtubules into the branch and the nucleation of microtubules within the new tip. Cortical complexes involved in microtubules' capture and nucleation presumably localize to the new tip and mediate these processes.

In addition, genetic analyses in *N. crassa* have identified a set of genes that are likely to be involved in microtubule function and are required for maturation of new lateral branches (Seiler and Plamann 2003). Notably, these genes (i.e. *pod-4*, *pod-5* and *pod-8*) are only required for branch maturation; they have no obvious role in morphogenesis of the primary hypha.

COMPARISON TO OTHER BRANCHING SYSTEMS

In addition to the filamentous fungi, other eukaryotic (i.e. Oomycetes) and prokaryotic (i.e. Streptomyces) organisms propagate via the formation of branched hyphal networks. The little that is known about the regulation of hyphal branching in these organisms already highlights similarities with fungi. For example, in bacterial hyphae, lateral branches appear to be positioned in proximity to septa, though nucleoids may also play a role in determining branch sites (Kretschmer 1992). In Oomycetes (i.e. *Saprolegnia ferax*), calcium appears to play a key role in the induction of hyphal branching, perhaps by promoting the local accumulation of "branch initiation factors" (Grinberg and Heath 1997). Additional studies are undoubtedly needed to determine the extent to which the mechanisms that underlie hyphal branching in these organisms parallel those used in fungi. Presumably, it might be possible to define a conserved sequence of events that lead to the emergence of a branch.

Plant and animal cells typically do not employ a branching mode of morphogenesis, though there are notable exceptions. Trichomes, or leaf hairs, are found on plant-cell surfaces, where they are thought to perform a variety of protective functions (Hulskamp 2004). In *Arabidopsis thaliana*, trichomes are single polyploid epidermal cells that undergo a stereotypical pattern of branching. Detailed genetic and molecular analyses have identified several key functions required for trichome branching (Schnittger and Hulskamp 2002, Hulskamp 2004), including local microtubule dynamics and Golgi body-related transport. Notably, branch sites appear to be determined using positional information provided by preceding cell-division events, which is reminiscent of the role that septa play in regulating hyphal branching in certain fungi. Animal neurons are single cells composed of multiple compartments, including a single axon and a highly branched dendritic arbor that extends from the cell body (Arimura and Kaibuchi 2005). The dendritic arbor enables a single post-synaptic neuron to receive inputs from multiple pre-synaptic neurons. A multitude of functions have been implicated in dendrite development, including

extracellular signaling molecules and a number of monomeric Rho GTPases (reviewed in Jan and Jan 2003). Although the analogy itself might be somewhat superficial, it is tempting to view the pre-fusion induction of hyphal branching by an adjacent tip as a process related to dendritic branching in response to neurotrophic factors. In this case, besides characterizing homologues of genes involved in the *S. cerevisiae* mating response (Glass et al 2004), it might also be prudent to search for homologues of genes involved in dendritic morphogenesis with a view toward testing their role in hyphal fusion.

FUTURE QUESTIONS

Mycologists have long recognized the importance of hyphal branching in the development of the fungal colony (Carlile 1995, Rayner et al 1995). So too have industrial microbiologists, who are well aware that branching is a critical determinant of colony morphology that ultimately affects the yield of fungal fermentations (e.g. Trinci 1994). Accordingly, considerable effort has been expended in an attempt to understand the critical temporal and spatial signals that trigger branching. As outlined in this review, important insights have been obtained using a variety of approaches. Moreover, with the development of post-genomic resources applicable to a diverse variety of fungi, the pace at which new insights emerge should accelerate over the next few years. Thus, it should soon be possible to address critical questions such as: (i) How are branch sites selected?; (ii) To what extent are the mechanisms underlying branch formation shared with germ tube emergence?; (iii) How is branch formation integrated with nuclear division, cellular growth and colony development?; (iv) Are the mechanisms that underlie branch formation universally employed across the fungal kingdom, or have different filamentous fungi evolved unique mechanisms that suit their particular life cycle?; (v) How do plants, algae and cyanobacteria subvert the branching process to initiate symbiotic partnerships with fungi? The answers to these questions should provide fungal biologists, metabolic engineers and other interested parties with a deeper understanding of how fungal colonies develop and should also reveal approaches that can be used to manipulate colony development for the benefit of human welfare.

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