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Review

The Spitzenkörper: a molecular perspective

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ARTICLE INFO

Article history:
Received 6 May 2005
Accepted 24 September 2005
Corresponding Editor: Teun Boekhout

Keywords:
Hyphal morphogenesis
polarized growth
Spitzenkörper
ultrastructure
vesicle supply centre

ABSTRACT

The Spitzenkörper is a dynamic structure present at the tips of hyphal cells with a single highly polarized growth site. It is closely connected with cell morphogenesis and polar growth, and is only present at actively growing sites. Morphogenesis of such highly polarized cells is complex, and requires the coordinated action of multiple protein complexes. We discuss the relevance of these complexes for the structure and function of the Spitzenkörper.

Introduction

Polarization of growth, a process of localizing growth to a discrete region of the cell, is a fundamental feature of fungal cellular physiology. It enables cells to assume a shape typical for the species, mate, and seek out shelter and nutrients. All organisms, whether unicellular or multicellular, utilize conserved core mechanisms to generate polarity (Nelson 2003). In addition, multicellular organisms generate polarized tissue by controlled cell divisions. Throughout kingdoms, organisms have evolved to form individual cells that select a single polarization site resulting in elongated, tubular shapes. Examples include neurons in animals (Andersen & Bi 2000), pollen tubes and root hairs in vascular plants (Hepler *et al.* 2001), rhizoids in algae (Kropf 1992), and true hyphae in fungi (Heath 1990; Gow 1995). A plethora of proteins and other compounds select, establish and maintain polarized sites (Sheu & Snyder 2001; Wiggin *et al.* 2005), and are addressed as polarity-related cellular components. Polarity-related cellular components are organized into functional interacting units such as the polarisome, the Arp2/3 complex, and the exocyst. The Spitzenkörper, a vesicle-organizing centre that is intimately involved in fungal cell morphogenesis, spatially and temporally coincides (at least partially) with polarity-related components. Therefore, it is useful to discuss them together. Our focus will be on highly polarized hyphal cells, as they are a group in which the Spitzenkörper can be found most frequently.

Spitzenkörper organization and dynamics

Fungi can be divided into those that make true hyphae, the filamentous fungi (e.g. *Aspergillus nidulans*, *Neurospora crassa*), and ones that either do not make true hyphae or make pseudohyphae, the yeasts (e.g. *Saccharomyces cerevisiae*,

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0953-7562/$ – see front matter
doi:10.1016/j.mycres.2005.09.005

Schizosaccharomyces pombe). One of the characteristic features for fungi with true hyphae is the presence of the Spitzenkörper, a highly dynamic cellular structure of variable composition and shape, without a membrane boundary, adjacent to the site of polarized cell extension. It is a structure present at actively growing tips that disappears when growth ceases. It also coincides with the direction of polarized growth (Brunswick 1924); the early literature is reviewed by Harris *et al.* (2005). As seen by light microscopy, the structure of the Spitzenkörper differs between species, but it is also dynamic and variable within a species (López-Franco & Bracker 1996; Dijksterhuis 2003). The most recent classification, based on structural observations using phase-contrast light microscopy, divides Spitzenkörper morphology in fungi into eight groups (López-Franco & Bracker 1996). Some elements, such as vesicles of various kinds, microfilaments, microtubules, and ribosomes, are generally present in the Spitzenkörper. However, not all of them are present in every species, or at all times in a single species. Furthermore, a single hyphal tip may have a variable Spitzenkörper composition through time.

For the hyphal tip to progress with growth, it must incorporate plasma membrane and cell wall constituents into the expanding apical surface. These constituents are packaged into vesicles, which are present without exception in every visible Spitzenkörper (Fig 1). The presence of vesicles in the Spitzenkörper was confirmed in fixed cells by ultrastructural studies using electron microscopy (Girbardt 1969; Grove *et al.* 1970). Studies of live cells using fluorescence, confocal microscopy and the amphiphilic styryl dye FM4-64, showed the dynamic nature of the vesicle accumulation in the Spitzenkörper (Fischer-Parton *et al.* 2000; Dijksterhuis 2003). Even before assessing experimental evidence, it is possible to predict that vesicles contain structural components of the plasma membrane and cell wall, landmark proteins, proteins that facilitate membrane docking and fusion, and signaling modules. Even though the content of vesicles in the Spitzenkörper is not clear due to technical difficulties associated with their differential isolation, some data have been collected. For example, a subset of apical vesicles are zymogenic chitin synthetase-containing vesicles termed chitosomes (Bracker *et al.*

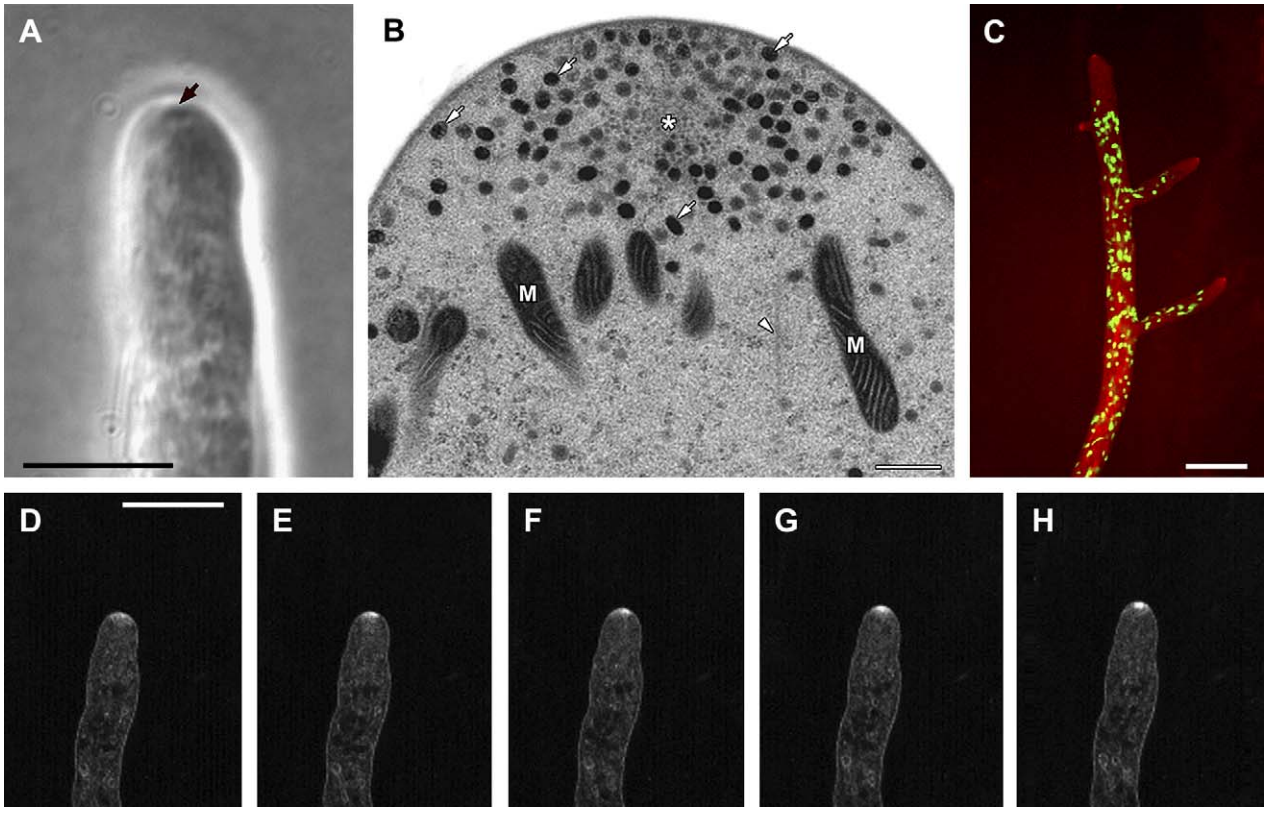

Fig 1 – Spitzenkörper in hyphal tips of *Neurospora crassa*. (A) Phase contrast image. The Spitzenkörper (black arrow) is visible as a dark more or less spherical region at the tip with a subtending light core region (pattern 8, as defined by López-Franco & Bracker 1996). Bar = 10 μm. The image was observed on an Olympus BX51 fluorescence microscope with phase contrast optics. (B) Transmission electron microscope image of a cryofixed and freeze-substituted hyphal tip. The Spitzenkörper is visible as a cloud of apical vesicles (arrows) surrounding a core (asterisk). Microtubules (arrowhead) and mitochondria (M) are also visible. Bar = 3.3 μm. From Riquelme *et al.* (2002) with permission. (C) Dual live staining of membranous material with FM4-64, and nuclei with H1-GFP (strains are available through the Fungal Genetics Stock Center, Freitag *et al.* 2004). The Spitzenkörper is visible as an accumulation of membrane-bound vesicles at the hyphal tips. Bar = 20 μm. Image was observed on a Zeiss Axioplan 2 Fluorescence microscope. (D-H) Dynamic changes in the Spitzenkörper size and shape during growth of a FM4-64 stained hyphal tip. Successive images were collected at 15 s intervals. Bar = 20 μm. Images were collected on a BioRad Radiance 200 Confocal Microscope.

1976; Sietsma *et al.* 1996). The Rab-GTPase Sec4 homologue (Punt *et al.* 2001) has also been identified in tip-located vesicles. In addition, calcium localization experiments (Torralba *et al.* 2001) and UV beam-mediated calcium release experiments (Grinberg & Heath 1997) suggest the presence of vesicles that serve for calcium storage. Other vesicles are proposed to have receptors that bind to signaling molecules such as inositol (1,4,5) phosphate (IP_3; Silverman-Gavrila & Lew 2002).

Ultrastructural studies showed the presence of microtubules and microfilaments in the Spitzenkörper (Grove & Bracker 1970; Howard 1981; Hoch & Staples 1983; Srinivasan *et al.* 1996). Material for new plasma membranes and cell walls is produced in the endoplasmic reticulum and processed in the Golgi tubules at sites distant from their final destination. From there it is transported in vesicles to the hyphal tips by the cytoskeleton and its associated motors (Grove *et al.* 1970). Therefore, the presence of cytoskeletal elements such as microtubules and microfilaments in the Spitzenkörper is not surprising, as it would be the site where the cytoskeleton releases vesicles at the tip.

Microtubule disruption results in a drastic reduction in hyphal tip growth, although growth still continues at a slow rate, implicating microtubules in rapid tip growth (Horio & Oakley 2005). Microtubules involved in apical growth are distinct from microtubules involved in mitosis, as the Spitzenkörper does not show changes in its presence during mitosis, and a subset of microtubules persist at the hyphal tip, when the rest of the microtubule population is disassembled (Riquelme *et al.* 2003; Horio & Oakley 2005). Microtubule-organizing centres (MTOC) are present throughout the hyphae (Straube *et al.* 2003; Konzack *et al.* 2005), and are associated with nuclear positioning (Plamann *et al.* 1994). It has been suggested that the Spitzenkörper serves as a MTOC, given its crucial position, and the necessity of microtubules for the transport of vesicles to the tip. However, γ-tubulin, which is characteristic for MTOCs, has only been found in the Spitzenkörper of *Allomyces macrogynus* to date (McDaniel & Roberson 2000). Two groups of motor proteins associated with microtubules shuttle vesicles and organelles in the cytoplasm: kinesins and the dynein/dynactin complex. Kinesins are responsible for plus end directed anterograde transport, while the dynein/dynactin complex is responsible for minus end directed retrograde transport (Goodson *et al.* 1997). The loss of kinesin function results in altered hyphal morphogenesis and failure to establish a Spitzenkörper (Seiler *et al.* 1997). As expected, hyphae in mutant strains with changes in the dynein/dynactin complex still have a prominent Spitzenkörper, while hyphae in a strain with a mutation in kinesin possess a reduced Spitzenkörper, or lack a visible Spitzenkörper (Seiler *et al.* 1999), indicating that plus ends of apical microtubules are mainly oriented towards the tip. However, later more detailed studies showed that dynein deficiency results in a reduced number of vesicles within the Spitzenkörper (Riquelme *et al.* 2002). These observations suggest that both retrograde and anterograde transport is necessary for hyphal morphogenesis and Spitzenkörper integrity although the effect of retrograde transport on the Spitzenkörper is indirect. In addition, KipA, an *Aspergillus nidulans* homologue of the Tea2 kinesin (Browning *et al.* 2003), affects the position and size of the Spitzenkörper (Konzak *et al.* 2005). In *Schizosaccharomyces pombe*, Tea2 is loaded on microtubule tips close to the nucleus and travels on polymerizing microtubules at their plus end (Browning *et al.* 2003). At polymerizing microtubule tips, Tea2 transports Tea1 to the apical cortex, where it is deposited at a site that appears to be marked by the Mod5 landmark protein (Feierbach *et al.* 2004). KipA was also proposed to be involved in anchoring microtubules to the cortex, suggesting that microtubules and associated proteins participate in anchoring the Spitzenkörper to the apex.

The necessity of actin for the integrity and function of the Spitzenkörper was demonstrated by treatment of hyphal tips with actin filament inhibitors (Heath *et al.* 2003), and characterizing actin distribution in actin mutants (Virag & Griffiths 2004). Disassembly of microfilaments in the cytoplasm results in the abortion of tip growth and the disappearance of the Spitzenkörper (Heath *et al.* 2003), while mutation in the actin gene results both in a modified actin distribution and a reduced Spitzenkörper size (Virag & Griffiths 2004). One of the main roles of tip-localized actin with regards to Spitzenkörper function is vesicle transport at the hyphal tip. The class I actin-associated motor protein MyoA is essential for polarized growth, associates with vesicles and localizes to the hyphal tip (McGoldrick *et al.* 1995; Yamashita *et al.* 2000), consistent with its proposed function as an actin motor protein transporting vesicles within the hyphal tip.

Ribosomes are present in the Spitzenkörper, thereby implying that localized intensive protein synthesis occurs at the hyphal tip (Grove & Bracker 1970). Together with microvesicles, microtubules, and microfilaments, ribosomes were proposed to comprise a Spitzenkörper core region. Other structures, such as filasomes, vesicles associated with filamentous material, have also been reported (Howard 1981). Ultrastructural immunocytochemistry in *Sclerotium rolfsii* indicated that filasomes are an ultrastructural equivalent of actin plaques (Roberson 1992).

Immuno-labeling in fixed cells and GFP labeling in live cells has further broadened the collection of proteins that localize to the Spitzenkörper region (see below). Highly polarized plant cells also show the presence of a Spitzenkörper. However, the structure is different than the fungal Spitzenkörper. The Spitzenkörper of *Chara* rhizoids, for example, contains an endoplasmic reticulum aggregate in addition to vesicles, a central actin array that radiates pronounced actin filaments in all directions, profilin, actin depolymerizing factor (Braun *et al.* 2004), and spectrin-like proteins (Braun 2001).

The Spitzenkörper and polarity establishment factors

An important issue that needs to be addressed in the context of polarity establishment and hyphal morphogenesis, is the ontogeny of the Spitzenkörper. Observations of dichotomous branching in the *Aspergillus niger* 'ramosa' mutant (Reynaga-Peña & Bartnicki-García 1997; Reynaga-Peña *et al.* 1997) showed that the Spitzenkörper disappears before the new branches of a dichotomous branch are formed. Two new Spitzenkörper are produced after the initials of the two new branches become visible, one at the tip of each new branch. Although it is possible that the core of the initial

Spitzenkörper persists and divides, there is no evidence to support this so far. Therefore, it is most likely that new Spitzenkörper are formed *de novo*. If this is true, the Spitzenkörper is not necessary for the selection of a new polarization site, but rather has a function in polarity maintenance. In support of *de novo* formation, satellite Spitzenkörper were reported to form at a short distance from the tip, before they merge with the main Spitzenkörper (López-Franco *et al.* 1995). This mode of feed-in to the Spitzenkörper was proposed to account for the inherent pulsed growth of hyphal tips in general (López-Franco *et al.* 1994). *De novo* formation also appears to be characteristic during lateral branching, as was demonstrated in *Neurospora crassa* (Riquelme & Bartnicki-García 2004).

Although the connection of the Spitzenkörper with polar growth is evident, the role of the Spitzenkörper in polar growth during hyphal morphogenesis is still not clear. The absence of the Spitzenkörper in hyphae that are not growing suggests the possibility that it is only a visible manifestation of the dynamic vesicle and organelle movements and movements of other cellular components at the hyphal tip. There are several Spitzenkörper characteristics that support this view. One is the diversity in the Spitzenkörper shape and composition within a single hypha through time, as observed by FM4-64 staining, and ultrastructural studies. Another is the position of the Spitzenkörper, which varies from being at the extreme apex to being slightly retracted from the tip. The Spitzenkörper can be observed at the extreme apex during fast growth, when the vesicle density at the apex is higher, while a retracted position can be observed at slower growth rates, or before the disappearance of the Spitzenkörper due to growth cessation or preparation for apical branching, when the vesicle density is lower. In this context, it is interesting that filamentous fungi that do not have a prominent Spitzenkörper, or do not have a Spitzenkörper at all, generally have slow hyphal growth rates. It could be argued that a more disperse and retracted Spitzenkörper would result in a wider hyphal diameter. However, a wider hyphal diameter does not need to be the consequence if we invoke yet another parameter—the rate at which the apical cell wall loses its plasticity. A decrease of cell wall plasticity would compensate for reduced vesicle influx.

On the other hand, the Spitzenkörper could be a structure actively assembled in the hierarchy of the relay process that transforms internal or external signals into a polarized growth response. The upstream components of this relay process could include the signaling protein Cdc42 (Johnson 1999) and other Rho-GTPases, including Rac-like proteins (Brown & Gow 2001; Burridge & Wennerberg 2004). During budding in *Saccharomyces cerevisiae*, proteins associated with the previous bud site provide positional cues for polarity establishment that recruit the signaling protein Cdc42 (Chant 1999). The Ras-like GTPase Bud1 mediates this recruitment. In a yeast that forms dichotomously branched hyphae, *Ashbya gossypii*, Rsr1/Bud1 is required for maintaining polarized hyphal growth (Bauer *et al.* 2004). During mating in *S. cerevisiae*, the highest pheromone concentrations are sensed and relayed through G-protein signaling pathways, also resulting in the localized accumulation of Cdc42 and its guanosine nucleotide exchange factor (GEF) Cdc24. Two more components, the GTPase activating proteins (GAP) Bem3 and Rga1, and the guanine nucleotide dissociation inhibitor (GDI) Rdi1 regulate the activity of Cdc42 (Schmidt & Hall 1998). The internal and external cues recognized by the Cdc42 signaling protein complex are then transmitted to the p21 activated kinases (PAKs), Cla4 and Ste20, and subsequently to their effectors that translate this information into a localized growth response (Pruyne & Bretscher 2000). In animal neurons Rho-family GTPases, including Rho, Rac, and Cdc42 are required for polarization as well (Horton & Ehlers 2003; Nelson 2003). They are proposed to recruit downstream elements that are different than in yeast cells, and include the Par3/Par6/aPKC complex. Par3 and Par6 are scaffold proteins that bind both Rho-family GTPases and atypical Protein Kinase C (aPKC), and are initially located at all tips (Horton & Ehlers 2003). Subsequently, those complexes are excluded from dendrites and maintained in axons. Although the mechanism by which this occurs is not clear, selective degradation or destabilization of mPar3 and mPar6 in all but the axon tip, or stochastic fluctuations of Par3 and Par6 that would increase the concentration of Par3 and Par6 in one of the tips by a positive feedback loop were suggested (Horton & Ehlers 2003). Proteins that are downstream effectors of the Par3/Par6/aPKC complex are not identified yet, although data suggest that polarity is regulated through microtubule function. In filamentous fungi homologues of the budding yeast cortical cues, such as Bud8 and Bud9, are not present, but homologues of Cdc42 and its regulators are (Fig 2; Harris & Momany 2004; Seiler & Plamann 2003). In addition, and in contrast to yeasts, Rac homologues seem to play an important role in polarity establishment, as shown in *Penicillium marneffei* and *Colletotrichum trifolii* (Boyce *et al.* 2003; Chen & Dickman 2004), but homologues to the Par3/Par6/aPKC complex are not identified. This suggests that although some upstream modules are conserved, the way in which they assemble and control polar growth may be unique in filamentous fungi. Adaptor proteins such as paxillin may also be important in the information relay. Paxillin is a multi-domain adaptor protein present at focal adhesions in cultured mammalian cells, and serves as a scaffold for recruiting signaling module and other proteins (Brown & Turner 2004).

Once a polarization site is selected, the machinery that enables and maintains polar growth is assembled. This is also the earliest point in the relay process where the Spitzenkörper may assume its role in polarized growth. The polarity maintenance machinery includes the cytoskeleton components that mediate transport to the hyphal tip, as well as a cortical network situated at the tip. The role of cytoskeleton components has already been discussed in the previous section concerning structural components of the Spitzenkörper. The cortical area of a *S. cerevisiae* polarized bud contains a group of proteins termed the polarisome located in the tip area just beneath the apical plasma membrane (Pruyne & Bretscher 2000). Members of the *S. cerevisiae* polarisome include Bni1, Pea2, Spa2, and Bud6. These proteins comprise a functional unit that directs cytoskeletal and other cell components towards sites of highly localized cell expansion. Spa2 serves as a scaffold protein that enables the proper assembly of the polarisome complex (Sheu *et al.* 1998; Pruyne & Bretscher 2000). Bni1 is a formin that binds to other polarisome components, as well as Rho-GTPases, transferring the

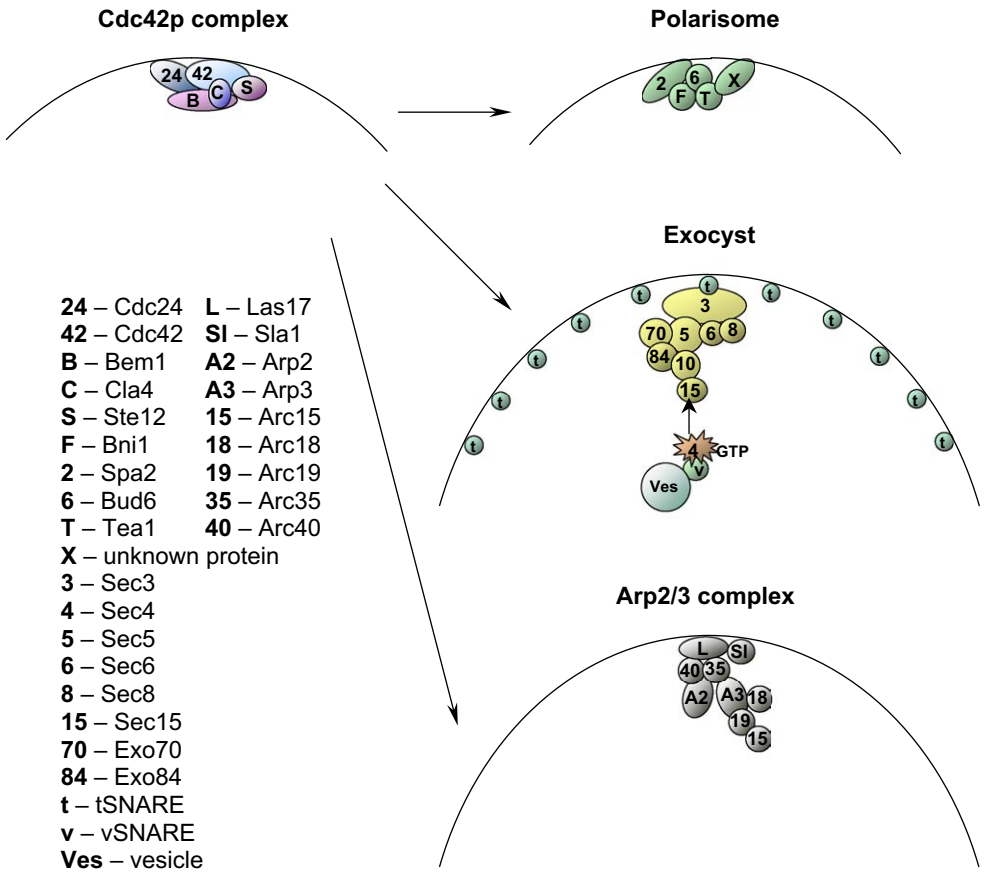

Fig 2 – Complexes with components important for polarity. The Cdc42 complex interacts with all the other presented complexes. See text for details.

signal relayed by the Cdc42 complex (Evangelista *et al.* 1997). Bud6 interacts with Bni1, and together they are responsible for the assembly of actin filaments (Evangelista *et al.* 1997). Although less is known about polarity components in filamentous fungi, it is clear that they differ in the presence of structural polarity-related cell components compared to non-filamentous yeasts. With an increasing number of fungal genomes being sequenced, it is possible to take a reverse genetics approach and search for polarity components in filamentous fungi. Homologues of the polarisome components Bni1, Spa2, and Bud6 are present (Fig 2), whereas Pea2 homologues are not (Harris & Momany 2004; Seiler & Plamann 2003). The absence of the *pea2* homologues and the presence of the rest of the polarisome components in all filamentous fungi suggest that there are differences in the control of polarized growth between filamentous and non-filamentous fungi. In *A. nidulans* the homologue of Bni1, SepA, was characterized and shown to be involved in maintaining polarity and in septum formation (Sharpless & Harris 2002). At the hyphal tip, SepA-GFP is visible at the apical membrane and as a dot behind the membrane. This dot-like distribution at the tip suggests that the formin may be a part of the Spitzenkörper. Recently, a novel protein unique for fungi, MesA, was shown to have a function in retaining SepA at polarization sites (Pearson *et al.* 2004). Two other components of the polarisome, the *spa2* homologue, *spaA*, and the *bud6* homologue, *budA* are currently in the process of being characterized (Virag & Harris, unpubl.). Preliminary results with fusion proteins show that SpaA localizes to the tips, while BudA localizes to septa, further supporting the view that the polarisome components and function may not be fully conserved in filamentous fungi. Thus it is still not clear whether filamentous fungi utilize a yeast polarisome equivalent.

In *Candida albicans*, the Spitzenkörper is present in hyphae, but not in pseudohyphae or yeast cells (Crampin *et al.* 2005). As *C. albicans* is a dimorphic fungus with the capacity to express bona fide yeast and hyphal morphologies, this indicates the selective use of subsets of available morphogenetic machineries for each of the morphologies, and confirms that the continued presence of the Spitzenkörper is unique and specific for true hyphae. Those authors also showed that the Spitzenkörper and polarisome are distinct entities with limited overlap, and that the polarisome components Spa2 and Bud6 are required for Spitzenkörper formation. The Spitzenkörper was identified in another yeast with true hyphae-*Ashbya gossypii* (Köhli *et al.* 2005). Here the spatial distribution of the *A. gossypii* Spa2 homologue, AgSpa2 suggests that this polarisome component colocalizes with the Spitzenkörper (Knechtle *et al.* 2003). However, the distribution of the *A. gossypii* homologue of Bni1, AgBni1, differs from the AgSpa2 localization, and is present at the tip cortex (Philippsen *et al.* 2005). Therefore, it is still not clear whether the distinction between

the Spitzenkörper and polarisome is unique for *C. albicans* or is a general rule, and more studies are necessary before conclusions can be made.

That 95 % of *A. gossypii* open reading frames have homologues in *S. cerevisiae* (Dietrich *et al.* 2004) brings up another interesting aspect of the presence of a Spitzenkörper in *A. gossypii*. It suggests that the organism likely utilizes components of existing machineries to control polarization events in radically different ways than *S. cerevisiae*. It also raises the question of how similar hyphal growth is in yeasts and filamentous fungi (Wendland & Walther 2005).

The presence of the Spitzenkörper in true hyphae but not pseudohyphae and yeast cells may be due to the necessity for efficient long-range transport of vesicles in true hyphae. Microtubules are prominent cytoskeleton components that serve as tracks for long-distance transport of vesicles of various content, and are clearly important for polarity maintenance in hyphae (Harris & Momany 2004). They are also often present in the Spitzenkörper, where they are proposed to unload their cargo before it gets distributed to its final destination (Harris *et al.* 2005), suggesting that their presence may be a pre-requisite for long-term Spitzenkörper maintenance.

The Arp2/3 complex is another set of proteins important for maintaining polarity, and includes Arp2, Arp3, Arc40, Arc35, Arc18, Arc19, and Arc15 (Fig 2; Mullins *et al.* 1997; Machesky & Gould 1999). The Arp2/3 complex is responsible for initializing branched actin filaments that form a cortical actin network and endocytosis. It localizes to patches adjacent to the plasma membrane in the apical region of the expanding hyphal tip. Components of this complex have not yet been found in the Spitzenkörper. However, activators of Arp2/3-dependent actin polymerization, such as members of the Wiscott-Aldrich Syndrome protein (WASP) family, are present at tips of polarized cells and interact with polarity components such as Cdc42 (Bompard & Caron 2004; Walther & Wendland 2004a,b).

Hyphal morphogenesis models and the Spitzenkörper

Several models exist that explain the interplay between regulatory and structural components of the Spitzenkörper during hyphal morphogenesis. However, each one emphasizes importance of specific aspects of tip growth.

The Vesicle Supply Center (VSC) model proposes the presence of a vesicle supply center at the hyphal tip that serves as a distribution center for plasma membrane and cell wall-containing vesicles (Bartnicki-García *et al.* 1990, 2000; Gierz & Bartnicki-García 2001). Vesicles are transported from distal parts of the cell to the VSC via the cytoskeleton, and are then randomly distributed to the surface where they fuse with the plasma membrane (Fig 3A). An apical microfilament-based network is involved in the transport of vesicles to their final destination. In this context, the Spitzenkörper can be perceived as a switching station where microtubule-based transport changes into microfilament-based transport (Harris *et al.* 2005). Bartnicki-García *et al.* (1990) propose that the Spitzenkörper is the structural equivalent of the VSC, as it occupies the area where the VSC is predicted to be. Two alternatives are put forward to explain continuous tipward displacement of the VSC: a pushing mechanism and a pulling mechanism. A pushing mechanism would include anterograde force resulting from an attachment of the VSC with cytoskeletal components, or from bulk flow of the protoplasm towards the tip. A pulling mechanism implies a link between the apical plasma membrane/cell wall and the VSC. The most likely candidates for a pulling mechanism are cell wall and plasma membrane interacting proteins such as integrins or spectrins that have

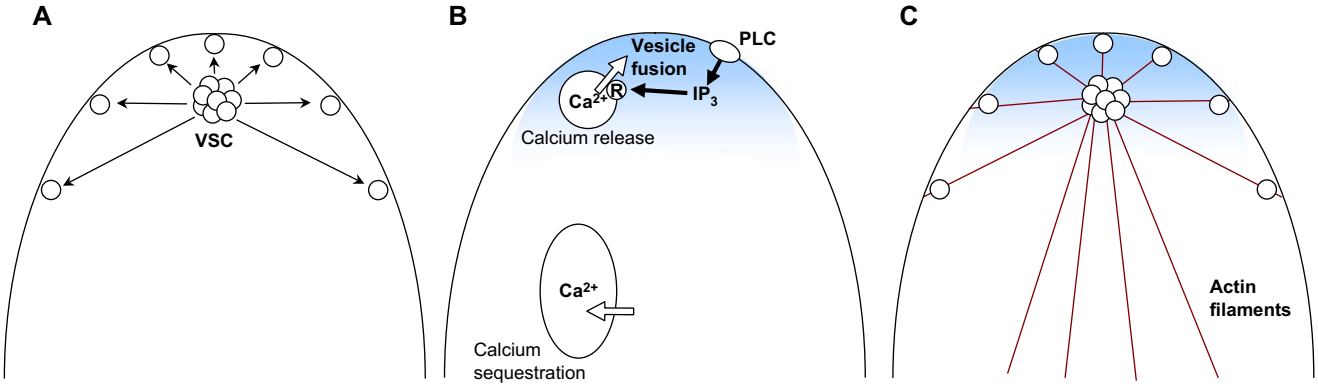

Fig 3 – Models for hyphal morphogenesis containing information relevant for the role of the Spitzenkörper in polarized growth. (A) The Vesicle Supply Center model. Vesicles are distributed randomly from an organized vesicle supply center (VSC) to their final destinations at the plasma membrane. The VSC is an equivalent of the Spitzenkörper. (B) The Calcium model. Stretch-activated phospholipase C produces the signaling molecule IP_3 that binds to receptors of calcium-containing vesicles and triggers the release of Ca^{2+} in the apical region. At sites distant from the hyphal tip Ca^{2+} is sequestered creating a tip-high Ca^{2+} gradient. High Ca^{2+} concentrations promote fusion of vesicles to the plasma membrane, resulting in the highest rate of incorporation at the extreme apex. PLC = phospholipase C. IP_3 = inositol (1, 4, 5) triphosphate. R = IP_3 receptor. Blue shading represents Ca^{2+} concentrations; the darkest shade corresponds to the highest concentration. (C) The Actin model. This model integrates the previous two models and, in addition, emphasizes the role of both apical and cytoskeletal actin in tip growth and Spitzenkörper maintenance.

actin-binding properties. However, other actin-binding or microtubule-binding proteins such as or Tea1p (Feierbach *et al.* 2004) are not excluded.

To understand how vesicles are distributed from the VSC to their final destination, it is first necessary to address how vesicle secretion is executed and regulated. Although few genes involved in vesicle secretion are cloned and analyzed in filamentous fungi, the mechanisms involved can be deduced by searching for gene homologues that contribute to vesicle secretion in other organisms. Filamentous fungi have homologues of members of the exocyst complex (Fig 2), as well as small GTPases from the Rab family of genes that regulate exocyst function (Punt *et al.* 2001; Seiler & Plamann 2003; Harris & Momany 2004). The exocyst complex is involved in vesicle docking and fusion to the plasma membrane (Lipschutz & Mostov 2002). This complex contains eight proteins: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84, and interacts with the Rho-GTPases Cdc42, Rho1, Rho3, as well as with Sec4, a Rab GTPase present on the surface of vesicular membranes (Lipschutz & Mostov 2002). A screen for mutants that contribute to polarized growth in *Neurospora crassa* revealed that a mutation in the Sec5 homologue of the exocyst complex results in swollen conidia and altered hyphal morphology, indicating its involvement in polarity establishment and maintenance (Seiler & Plamann 2003), as expected. A homologue of one of the genes from the Rab GTPase family, Sec4, was cloned and analyzed in *Aspergillus niger* (Punt *et al.* 2001). In yeast, this vesicle membrane-associated Rab GTPase interacts with the exocyst component Sec15 and activates the exocyst complex. However, in *A. niger*, its absence results in mild phenotypic changes, suggesting functional redundancy (Punt *et al.* 2001). This is supported by the observation that filamentous fungi have additional Rab GTPases compared to *Saccharomyces cerevisiae*. Alternatively, there may be important differences in the mechanisms that regulate exocyst function (Punt *et al.* 2001). Soluble N-ethylmaleimide-sensitive fusion protein (NSF) attachment protein receptors (SNAREs) are another category of proteins that facilitate the fusion between vesicles and target membranes (Gupta & Heath 2002; Burri & Lithgow 2004). As each membrane type has a specific SNARE, and only particular SNAREs can interact, SNAREs confer specificity upon the fusion process (Gupta & Heath 2002). At the hyphal tip, plasma membrane t-SNAREs presumably pair with vesicle membrane v-SNAREs (Fig 2). SNAREs are present in filamentous fungi, and two plasma membrane t-SNAREs, *nsyn1* and *nsyn2*, are characterized in *N. crassa* (Gupta *et al.* 2003). Although v-SNAREs associated with vesicles are likely to be present in the Spitzenkörper, this remains to be shown.

Harold (2002) pointed out another important aspect of the VSC model. He emphasized that even when not much is known about a process at the molecular level, an approach based on cell physiology can help to clearly present the principles of the process under investigation. The VSC model provides us with an explanation for the basic principles of hyphal morphogenesis. This ''top down'' approach (rather than the ''bottom up'' approach used by molecular biologists and geneticists) provides an alternative for vesicle incorporation at the hyphal tip, as well as how unusual branching patterns may arise, by invoking reaction-diffusion theory. Reaction-diffusion theory proposes that patterns in development can be generated from random fluctuations of chemical substances (Harrison *et al.* 2001). Crucial for the process is the appearance of at least two chemical substances termed morphogens, with properties of non-uniform distribution in space, nonlinear dynamics of their interactions, and transport by diffusion. Interactions between the two morphogens ultimately result in the formation of developmental patterns such as dichotomous branching, as seen in algae of the genus *Micrasterias* (Holloway & Harrison 1999), or lateral branching. The advantage of this approach is that it is possible to discuss the principals of hyphal development without knowing what the morphogens are. The pattern formed on the tip surface would direct the incorporation of vesicles at the selected site. Candidates for morphogens include Cdc24, Cdc42, cytoplasmic calcium, as well as other proteins involved in polarity that are located at the hyphal tip.

Another model emphasizes the role of Ca^{2+} concentrations at the hyphal tip (Fig 3B). The base for this model is the presence of a tip-high concentration of cytoplasmic and membrane-bound calcium at the hyphal apex (Shaw & Hoch 2001). This tip-high gradient is thought to be important for apical dominance (Schmid & Harold 1988). When this gradient is disrupted, apical growth becomes aberrant, leading to the formation of multiple tips or the complete loss of hyphal polarity. According to this model, stretch-activated phospholipase C activates the generation of inositol (1, 4, 5) triphosphate (IP_3) that then binds to IP_3 receptors on the surface of a subset of calcium-containing vesicles (Silverman-Gavrila & Lew 2002). The local increase of free Ca^{2+} in turn facilitates the fusion of vesicles with the plasma membrane, resulting in the highest incorporation at sites of highest Ca^{2+} concentration. Although this model does not dispute the presence of a Spitzenkörper at the tip or discuss the role of the Spitzenkörper, it gives an alternative to how vesicles are directed to the correct site of incorporation after the cytoskeletal elements release them.

A recent model integrates the preceding models and extends the role of actin at the hyphal tip (Virag & Griffiths 2004). This model emphasizes the dual role for actin filaments at the growing hyphal apex (Fig 3C). The two roles were teased apart by comparing hyphal morphogenesis and actin distribution in wild type and an actin mutant of *N. crassa*. As a component of the cytoskeleton, microfilaments control the rate at which vesicles travel to the tip. By doing this, they contribute to the maintenance of the calcium gradient. On the other hand, actin filaments are likely involved in anchoring the Spitzenkörper to the plasma membrane and cell wall. The lack of the connection between the two categories of actin filaments disrupts Spitzenkörper positioning in the actin mutant, which reflects on hyphal development and morphology.

All these models are developed to better understand the process of polarized growth. However, a model that will most accurately represent the intricacies of the process is yet to be developed, and will have to incorporate all the structural and regulatory components, as well as their mode of coordination at a higher, cellular level.

Conclusions

Clarification of several points is crucial to better understand the role of the Spitzenkörper. It is necessary to reveal the

contents of vesicles that travel to the tip, as this is important with regards to the fate of the vesicle. It is almost certain that there are different categories of vesicles that transport different material to the hyphal tip. Another point will be to establish the connections between structural components of polarity establishing complexes, such as the polarisome, and the Spitzenkörper. The ways in which the Spitzenkörper is assembled and regulated also need to be elucidated.

In conclusion, the Spitzenkörper is a dynamic structure present at sites of highly polarized growth during hyphal morphogenesis. Its composition is variable through time, and between species. The Spitzenkörper is associated with cellular functions necessary for cell wall formation. These cellular functions include organization and transport of components destined for incorporation into expanding sites, as well as assembly of elements involved in the regulation of polarized growth. The challenge in the future will be to characterize the intricate interplay between constituents of the Spitzenkörper and other polarity related complexes, and the way in which the Spitzenkörper responds to external and internal cues.

Acknowledgements

We would like to thank the BioImaging Facility at the University of British Columbia for use of their facilities for preparing the confocal images for Fig 1. We also thank Peter Sudbery for providing data prior to publication.

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