The Woronin Body: A Fungal Organelle Regulating Multicellularity

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CONTENTS

I. Multicellularity of Filamentous Fungi

Filamentous fungi form straight hyphae via polarized tubular extension of the hyphal tip and consist of a network of straight primary hyphae with the formation of branches. Hyphae are compartmentalized into distinct cells by the formation of a septum, which is not frequently observed in early-diverging fungi, such as members of the phyla *Mucoromycota* and *Chytridiomycota*, but is regularly found in later-diverging fungi belonging to the phyla *Ascomycota* and *Basidiomycota* (Jedd 2011). The septum is proposed to increase the mechanical integrity of hyphae and to divide the mycelium into sections of distinct growth states, such as mitotic and non-mitotic cells (Fiddy and Trinci 1976; Momany et al. 2002; Nayak et al. 2010;

Edgerton-Morgan and Oakley 2012). However, the septum does not completely separate hyphae due to the presence of a septal pore, which is a perforated structure that allows the exchange of the cytoplasmic constituents, including organelles, between adjacent hyphal cells (Lew 2005; Tey et al. 2005; Ng et al. 2009; Bleichrodt et al. 2015). Such cell-to-cell connectivity resembles gap junctions in animal cells and plasmodesmata in plant cells.

Cell-to-cell connectivity through the septal pore is associated with the catastrophic risk of cytoplasmic loss by cells adjacent to individually damaged hyphae. This risk was demonstrated by the physical damage of hyphae with a razor blade (Trinci and Collinge 1974) or pulse laser (Lichius et al. 2012), treatment with cell wall-destabilizing reagents (Bowman et al. 2002), and exposure to hypotonic shock (Fig. 1a) (Jedd and Chua 2000; Maruyama et al. 2005). Cell death is induced by aging and heterokaryon incompatibility (Fleißner and Glass 2007). Despite such damage, the cytoplasm of cells immediately adjacent to the wounded cell is typically retained (Fig. 1b) (Jedd and Chua 2000; Maruyama et al. 2005). The protected cells then initiate regrowth of the mycelium by producing a new hyphal tip (Jedd and Chua 2000; Maruyama et al. 2006; Maruyama and Kitamoto 2007; Lichius et al. 2012). These processes represent an inherent defense system for promoting survival by preventing the simultaneous loss of cytoplasm from multiple cells upon hyphal wounding. Hence, the septal pore is an important subcellular structure for maintaining the multicellularity of filamentous fungi.

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A

Hypotonic
shock
→

B

2nd cell   1st cell   2nd cell   1st cell
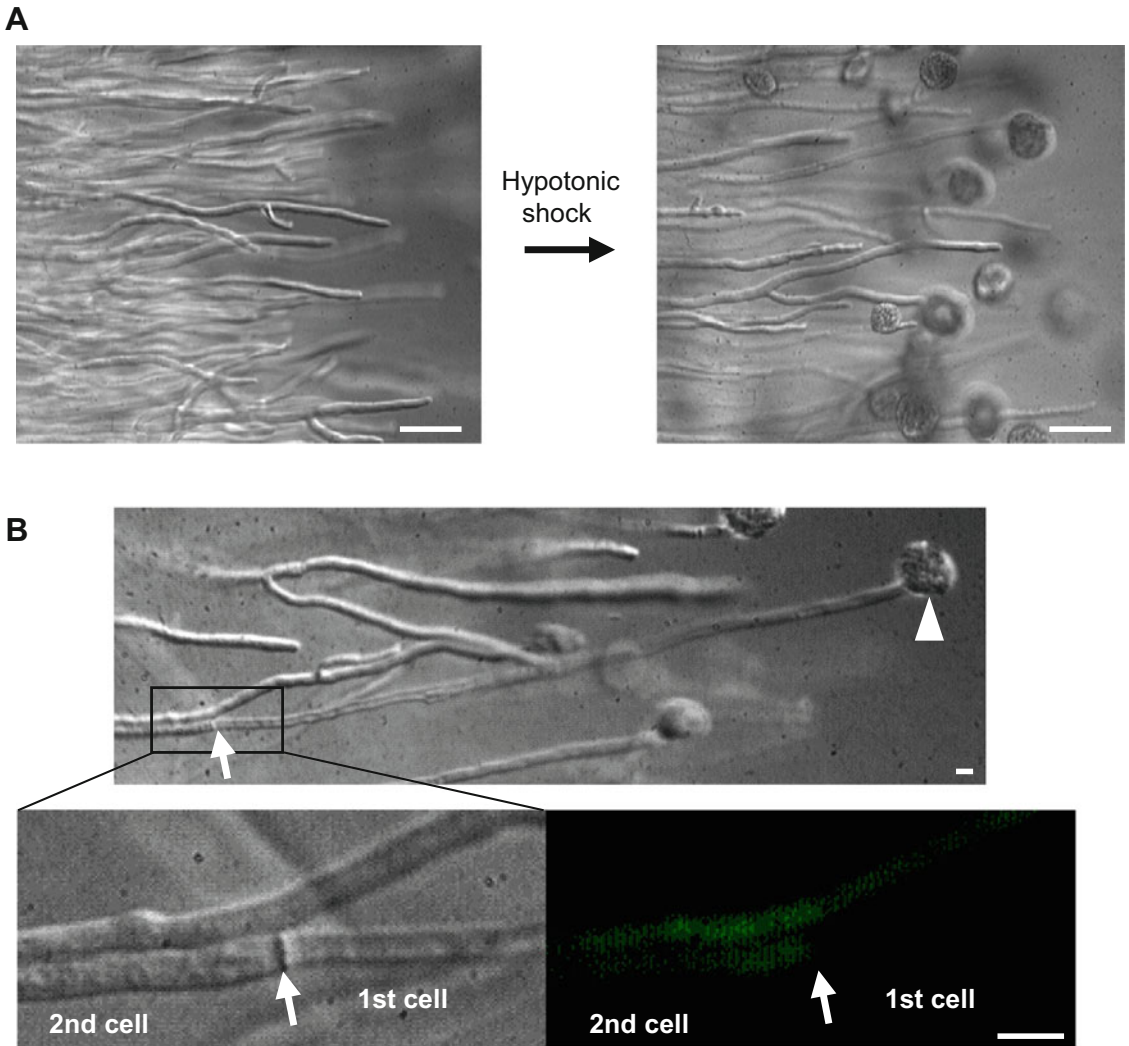
Fig. 1 Hyphal wounding and the prevention of excessive cytoplasmic loss. (**a**) Hyphal tip bursting upon hypotonic shock. Hyphal tips at the margin of an *A. oryzae* colony grown on agar medium were observed by differential interference contrast (DIC) microscopy before and after flooding hyphae with water. Bar: 50 μm. (**b**) Prevention of the excessive loss of cytoplasm upon hyphal wounding. The cytoplasm is labeled by expressing EGFP. An arrowhead and arrow indicate a burst hyphal tip and the adjacent septum, respectively. Note that the cell (2nd) adjacent to the lysed cell (1st) retains its cytoplasmic constituents, as determined by DIC and fluorescence microcopy. Bar: 10 μm

II. The Woronin Body, an Organelle Specific to *Pezizomycotina*

Filamentous fungal species that form septa have evolved to possess specialized membrane-bound organelles located around the septum; the *Pezizomycotina* (*Ascomycota*) contain structures called the **Woronin body** (Markham and Collinge 1987), and *Agaricomycotina* (*Basidiomycota*) have an endoplasmic reticulum (ER)-related septal pore cap (Müller et al. 1998). Woronin bodies were first identified as highly refractive particles located near the septum in the filamentous ascomycete *Ascobolus pulcherrimus* by the Russian mycologist Michael Stepanovitch Woronin (Woronin 1864). The organelle was later named by Buller

A

B
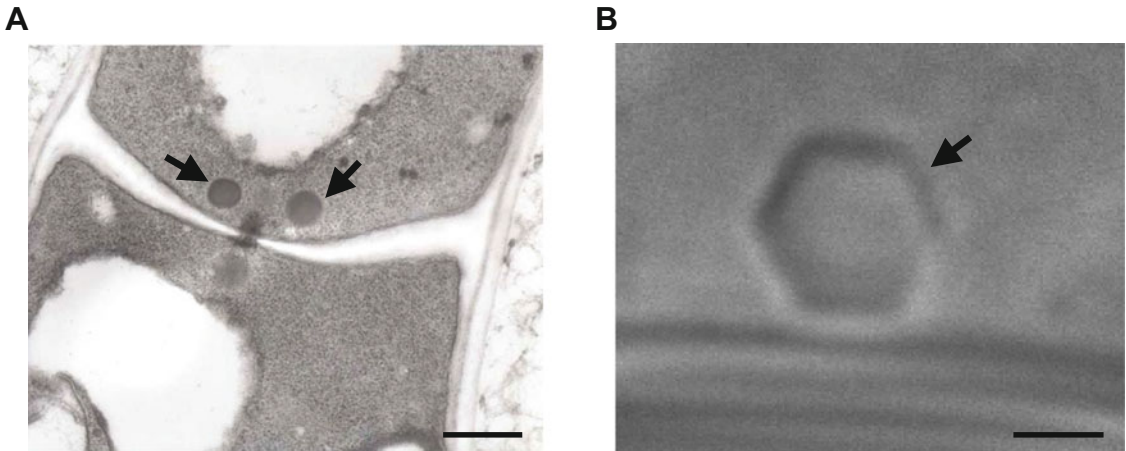
Fig. 2 Morphology of the Woronin body. (**a**) Transmission electron microscopic observation of Woronin bodies (arrows) near the septum in *A. oryzae*. Bar: 500 nm. (**b**) Differential interference contrast (DIC) image of a hexagonal Woronin body localized to the cell cortex in *N. crassa*. Woronin bodies are indicated by arrows. Bar: 1 μm

(1933) in recognition of Woronin's discovery and is restricted to *Pezizomycotina* species (Markham and Collinge 1987). The Woronin body has two morphologically distinct subclasses; it predominantly appears by transmission electron microscopy as a spherical electron-dense structure near the septum (Fig. 2a), although in a small number of species, such as *Neurospora crassa*, Woronin bodies form hexagonal crystalline structures that

are occasionally visible by light microscopy (Fig. 2b) (Markham 1994). By both electron and light microscopy, Woronin bodies were observed to plug the septal pore upon hyphal wounding (Markham and Collinge 1987). Therefore, it was long suspected that the primary function of Woronin bodies was the prevention of excessive cytoplasmic loss from cells adjacent to damaged or lysed cells (Fig. 3a).

A

B
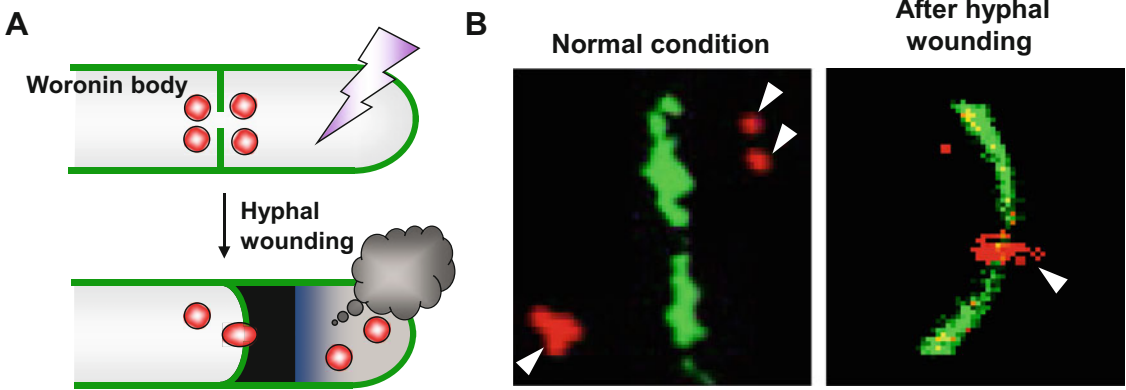
Fig. 3 Woronin body function. (**a**) Schematic representation of the septal plugging function of Woronin bodies. (**b**) Confocal images of Woronin bodies (red, arrowheads) and septa (green) before (left) and after (right) hyphal wounding. Woronin bodies and septa were labeled by DsRed2 and EGFP, respectively (Maruyama et al. 2005). Bar: 2 μm

III. Molecular Basis of Woronin Body Structure

Although Woronin bodies were discovered approximately 150 years ago, the composition of Woronin bodies long remained unclear. Protease digestion of ultrathin sections suggested that Woronin bodies contained proteinaceous materials (McKeen 1971; Mason and Crosse 1975). Woronin bodies from *N. crassa* were first purified in 2000 through differential and density gradient centrifugation, allowing for the identification of **Hex1** as a **major structural protein** (Jedd and Chua 2000; Tenney et al. 2000). Genes encoding Hex1 are conserved in *Pezizomycotina* species (Jedd and Chua 2000; Asiegbu et al. 2004; Curach et al. 2004; Soundararajan et al. 2004; Maruyama et al. 2005; Beck and Ebel 2013). Deletion of the *hex1* gene results in defective Woronin body formation and severe cytoplasmic bleeding upon hyphal wounding (Jedd and Chua 2000; Maruyama et al. 2005; Beck and Ebel 2013). When the apical neighboring cell is selectively ruptured, the subapical cells reinitiate hyphal growth by producing a new branch, but deletion of the *hex1* gene severely reduces or completely loses the activity of growth reinitiation (Tegelaar and Wösten 2017).

Characterization of Hex1 protein revealed that it spontaneously self-assembles to form a solid core, thereby providing mechanical resistance against the protoplasmic streaming pressure that is generated upon hyphal wounding (Jedd and Chua 2000; Yuan et al. 2003). The crystal structure of Hex1 consists of three intermolecular contacts that promote self-assembly (Yuan et al. 2003). Interestingly, the structural properties of Hex1 resemble those of eukaryotic translation initiation factor 5A (eIF5A) proteins (Kim et al. 1998; Peat et al. 1998), suggesting that the *hex1* gene evolved from *eIF-5A* by gene duplication in the ancestor of *Pezizomycotina*. Phosphorylation of Hex1 also contributes to the formation of the multimeric core of the Woronin body (Tenney et al. 2000; Juvvadi et al. 2007).

The *N. crassa hex1* gene encodes a single translational product (Jedd and Chua 2000), although alternative splicing of the *hex1* gene was reported in several species, including *Magnaporthe grisea*, *Aspergillus oryzae*, and *Aspergillus fumigatus* (Soundararajan et al. 2004; Maruyama et al. 2005; Beck et al. 2013). Both spliced and non-spliced transcripts yield two polypeptides that sediment in high-density protein fractions (Maruyama et al. 2005), suggesting that these peptides participate in the self-assembly and formation of the Woronin body core matrix. Moreover, it was demonstrated that a poly-histidine motif encoded within an alternatively spliced region targets Hex1 to the septal pore (Beck et al. 2013).

Although the hexagonal crystal structure of the *N. crassa* Woronin body is easily observed by light microscopy, the fusion of Hex1 with a fluorescent protein enables the septal plugging activity of Woronin bodies to be visualized in most *Pezizomycotina* species (Fig. 3b) (Maruyama et al. 2005; Beck and Ebel 2013). Using this approach, Bleichrodt et al. (2012) reported that the Woronin body reversibly plugs the septal pore during normal growth, a characteristic that contrasts the conventional view of this organelle functioning in wound healing. The analysis of gene expression activity for individual hyphae revealed that although wild-type cells exhibited heterogeneous activity, cells lacking Woronin bodies through deletion of *hex1* (Δ*hex1* strain) displayed more uniform gene expression activity (Bleichrodt et al. 2012). Thus, it was proposed that Woronin bodies contribute to the generation of hyphal populations with different cellular activities by occasionally preventing cell-to-cell connectivity via the plugging of the septal pore. The generation of such colonial heterogeneity under normal growth conditions may protect cells against environmental stresses, as evidenced by the sensitivity of the *A. oryzae* Δ*hex1* strain to heat stress (Bleichrodt et al. 2012). Thus, it appears that Woronin bodies have a gatekeeper role in regulating cell-to-cell channels by simple plugging of septal pore, similar to that observed upon hyphal wounding (Jedd and Pieuchot 2012).

In addition to septal plugging, Hex1 has other physiological impacts at the cellular level. For example, deletion of the *hex1* gene results in defective conidiation

(asexual spore formation) (Yuan et al. 2003; Son et al. 2013), impaired growth under nitrogen starvation (Soundararajan et al. 2004), and increased sensitivity to cell wall and membrane-destabilizing agents (Beck et al. 2013). In plant pathogenic fungi, Hex1 is also required for efficient pathogenesis and viral RNA accumulation (Soundararajan et al. 2004; Son et al. 2013). These lines of evidence suggest additional physiological functions of Woronin bodies, which need further investigation of the action mechanisms.

IV. Woronin Body Biogenesis from Peroxisomes

Electron microscopic studies examining the subcellular origin of Woronin bodies demonstrated the inclusion of these organelles within **microbodies** (Wergin 1973; Camp 1977). This finding was supported by the specific binding of antibodies against microbody-specific signal peptides to Woronin bodies (Keller et al. 1991). A relationship between peroxisomes and the Woronin body was clearly indicated by the finding that the C-terminus of Hex1 protein contains peroxisomal targeting signal sequence 1 (PTS1) (Jedd and Chua 2000). In addition, time-lapse imaging analysis revealed that Woronin bodies bud from peroxisomes (Tey et al. 2005).

Woronin body formation occurs at the hyphal apex through a process involving apically biased expression of the *hex1* gene (Tey et al. 2005). Woronin body biogenesis requires peroxins that mediate the import of peroxisomal matrix and membrane proteins (Fig. 4a) (Managadze et al. 2007; Liu et al. 2008; Li et al. 2014). Specifically, Hex1 associates with Pex26, a peroxin that is enriched in Woronin body-containing peroxisomes and recruits the AAA ATPases Pex1 and Pex6 to the peroxisomal membrane for receptor recycling (Liu et al. 2011). The recruitment of Pex26 leads to the formation of a parallel activation loop for Pex5 recycling and Hex1 import, allowing for the efficient biogenesis of Woronin bodies. Fam1 was originally identified in *Colletotrichum orbiculare* as a *Pezizomycotina*-specific ortholog of Pex22, which functions in the recycling of PTS receptors from peroxisomes to the cytosol (Fig. 4a).

Fam1 is specifically localized on the membrane of Woronin bodies, raising the possibility that recycling of PTS receptor occurs in Woronin bodies (Kubo et al. 2015).

In addition, the budding of Woronin bodies from peroxisomes appears to require dynamin-related proteins (Würtz et al. 2008). The peroxisome proliferator protein Pex11 is needed for the differentiation of Woronin bodies from peroxisomes (Fig. 4b) and also affects the septal plugging function of these organelles (Escaño et al. 2009). The genetic screening of *N. crassa* mutants defective in Woronin body biogenesis identified several peroxins and the Woronin body sorting complex (WSC) protein that were critical for this process (Liu et al. 2008). WSC is a *Pezizomycotina*-specific protein that recruits the Hex1 assembly to the matrix side of the peroxisomal membrane and facilitates budding of the Woronin body (Fig. 4b) (Liu et al. 2008). WSC belongs to the PMP22 (peroxisome membrane protein)/MPV17 (myeloproliferative leukemia virus 17) gene family and is proposed to have evolved to possess new functional properties, including self-assembly and Hex1 binding, from ancestral PMP22 (Jedd 2011).

ApsB, a component of the microtubule-organizing center (MTOC) that has functional peroxisomal targeting signal sequence 2 (PTS2), interacts with Hex1 (Zekert et al. 2010). In addition, TmpL, a transmembrane protein involved in redox-related signal transduction, is localized to Woronin bodies (Kim et al. 2009). However, it is unclear how ApsB and TmpL are involved in Woronin body biogenesis and function.

V. Septal Tethering of the Woronin body

In most *Pezizomycotina* species, Woronin bodies are typically tethered to the septum by a filament at a distance of 100–200 nm (Momany et al. 2002; Maruyama et al. 2005). The tethering structure was shown to have elastic properties, as demonstrated by the rapid return of the Woronin bodies to the septum after physical separation by laser trapping (Berns et al.

A

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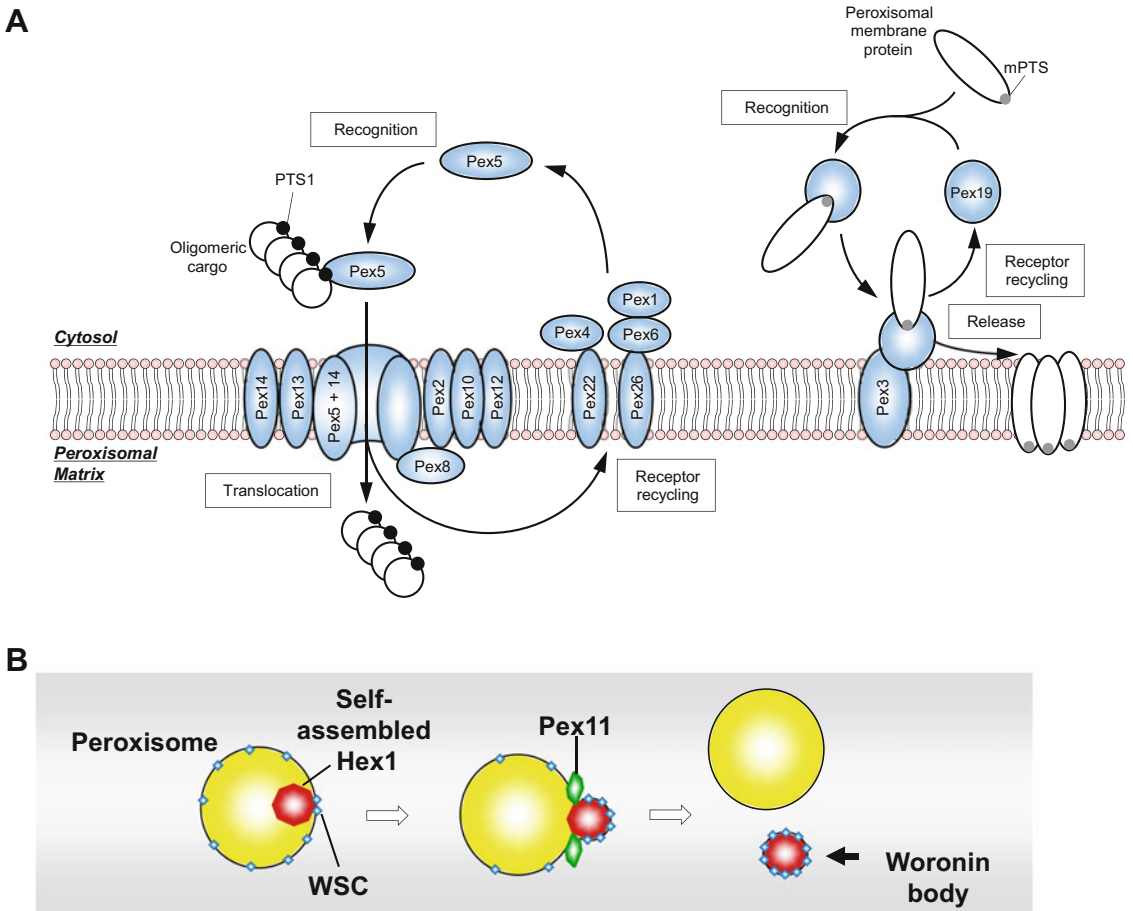
Fig. 4 Woronin body biogenesis from peroxisomes. (**a**) Schematic diagrams of the import machineries of peroxisomal matrix and membrane proteins. Peroxins labeled in blue were reported to be involved in

Woronin body function. *PTS1* peroxisomal targeting signal 1, *mPTS* membrane peroxisome targeting signal. (**b**) Model of Woronin body biogenesis from peroxisomes

1992). Moreover, Woronin bodies rapidly insert into the septal pore upon hyphal wounding and also reversibly plug the septal pore during normal growth (Bleichrodt et al. 2012). These processes require the proper positioning of the Woronin body and sufficient flexibility of the tethering linker.

In contrast, the Woronin bodies of a few members of the genera *Neurospora* and *Sordaria* are associated with the cortex in a delocalized pattern (Plamann 2009). In *N. crassa*, newly synthesized Hex1 proteins are imported into peroxisomes in the apical compartment (Tey et al. 2005), and the newly formed Woronin bodies are then inherited into subapical

compartments via association with the cell cortex (Tey et al. 2005; Liu et al. 2008). During a screening for mutants that accumulate Woronin bodies in the apical compartment, Ng et al. (2009) identified the *leashin* locus, which is comprised of the adjacent genes *lah-1* and *lah-2*. The N-terminal region of LAH-1 binds to Woronin bodies via the membrane protein WSC, whereas the C-terminal region mediates the association of Woronin bodies with the cell cortex (Fig. 5a) (Ng et al. 2009). In contrast, LAH-2, which localizes to the hyphal tip and septum, is not involved in Woronin body function (Fig. 5a). In *N. crassa* cells expressing an LAH-1/LAH2 fusion construct, Woronin bodies
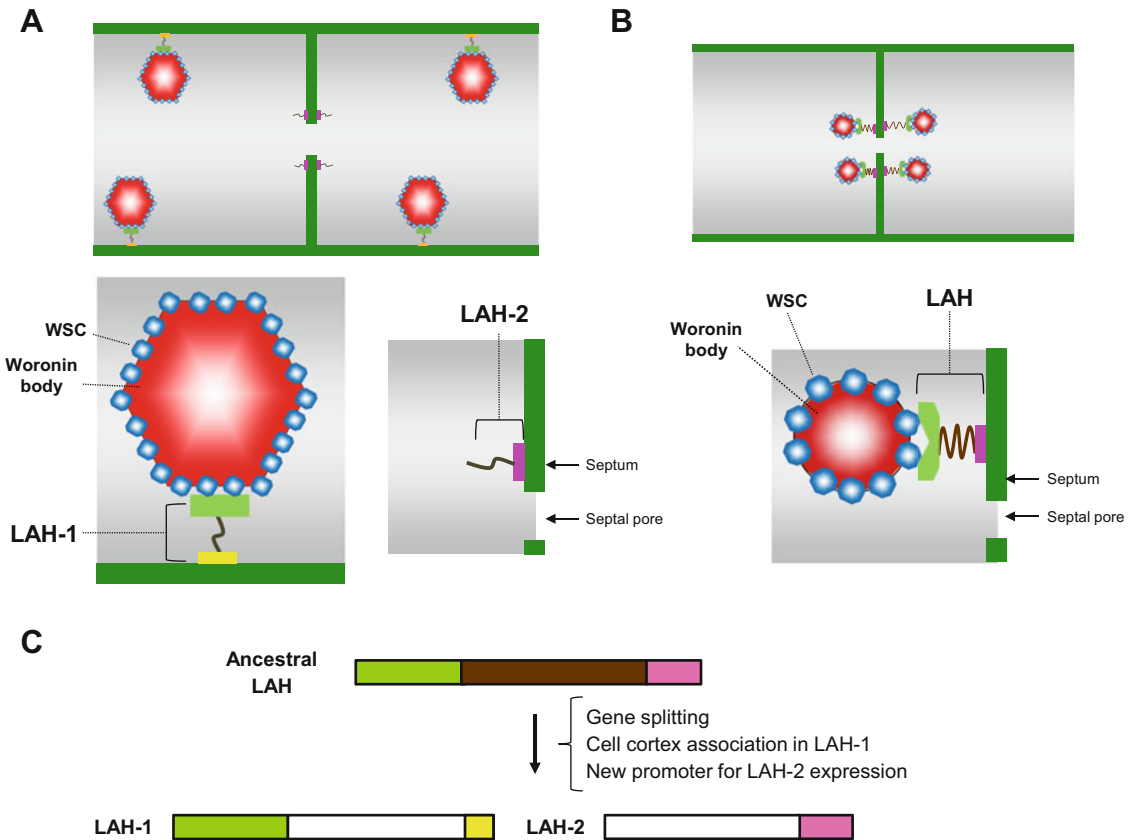
Fig. 5 Subcellular localization of Woronin bodies and tethering proteins. (**a**) Delocalized pattern of cortex-associated Woronin bodies via LAH-1 protein. (**b**) Sep-tal tethering of Woronin bodies via LAH protein. (**c**) Illustration of the generation of two LAH proteins from the ancestral LAH protein

accumulate at both sides of the septum, which only has partial ability to prevent the excessive loss of cytoplasm upon hyphal injury (Ng et al. 2009). This finding indicates an additional requirement of the tethered Woronin bodies for **septal pore plugging**.

Other species, such as *A. fumigatus* and *A. oryzae*, with tethered Woronin bodies have large **LAH proteins** consisting of a single poly-peptide of over 5000 amino acids (Beck et al. 2013; Han et al. 2014). LAH is required for the tethering of Woronin bodies to the septum and is involved in the Woronin body function of preventing the excessive loss of cytoplasm but to a lesser extent than the major Woronin body protein Hex1 (Han et al. 2014). This property may be explained by the fact that untethered Woronin bodies are able to plug the septal pore, but not as quickly as tethered ones.

Large LAH protein can be functionally divided into conserved N- and C-terminal regions and a non-conserved central region (Beck et al. 2013; Han et al. 2014): The N-terminal region associates with Woronin bod-ies in a WSC-dependent manner, and the C-terminal region containing a transmembrane spanning region mediates localization of LAH to the septum (Fig. 5b) (Beck et al. 2013; Han et al. 2014; Leonhardt et al. 2017). A truncated LAH protein consisting of only the N- and C-terminal regions retains the ability to tether Woronin bodies to the septum; however, the Woronin bodies are located closer (~50 nm) to the septum than those in wild-type cells (Han et al. 2014). This difference is roughly consistent with the 70-nm length of the approx-imately 2700-amino-acid central region of LAH, as was estimated based on the length

(1 μm) of the 4-mDa protein titin (Nave et al. 1989). The elasticity of the Woronin body tether that was previously demonstrated by laser-capture experiments (Berns et al. 1992) is conferred by the central non-conserved region of LAH (Han et al. 2014). The non-conserved central region in LAH is predicted to be disordered (Han et al. 2014) and presumably functions as a molecular spring, similarly to the muscle protein titin, which exhibits molecular spring-like elasticity via its intrinsically disordered region (Li et al. 2001). Moreover, in cells expressing LAH protein lacking the central region, tethered Woronin bodies do not plug the septal pore, even after hyphal wounding (Han et al. 2014). Collectively, efficient septal plugging requires not only that Woronin bodies are tethered to the septum but also that the tether must have sufficient elasticity to allow for the relatively unrestricted movement of Woronin bodies. The occasional observation that Woronin bodies from the ruptured cell side plug the septal pore suggests an active mechanism of Woronin body movement (Steinberg et al. 2017a). Surprisingly, ATP depletion by the respiration inhibitor carbonyl cyanide *m*-chlorophenylhydrazine induces the translocation of Woronin bodies into the septal pore without cell wounding. This suggests that ATP is required to prevent the septal plugging by Woronin bodies, leading to the speculation that ATP may bind to the tethering protein LAH for preventing a conformational change and contraction of the protein (Steinberg et al. 2017b).

The molecular origin of LAH remains elusive, but this protein first appeared in the clade of *Pezizomycotina* species together with Woronin bodies (Jedd 2011). The ancestral *lah* gene encodes a single, large polypeptide and was subsequently split into two genes, *lah1* and *lah2*, in the *Neurospora-Sordaria* clade. The *lah1* gene acquired an early termination sequence and a new cortex association domain at the C-terminus and a new promoter controlled expression of the downstream *lah2* gene (Fig. 5c). Thus, as a result of the gene splitting of the *lah* gene, the localization of the Woronin body was shifted from septal tethering to the cortex. *N. crassa* and *Sordaria fimicola* exhibit extensive cytoplasmic streaming through septal pores (Lew 2005; Tey et al. 2005; Ng et al. 2009) and have unusually rapid growth rates (>1 μm/s) (Ryan et al. 1943). As the tethering of

Woronin bodies to the septum may have reduced the exchange of cytoplasm between adjacent cells, a delocalized pattern of Woronin bodies with cortex association may have been selected to support the rapid growth of these species.

VI. Proteins Associated with the Woronin Body

Through the purification of Woronin body-associated proteins and bioinformatics approaches for detecting proteins that contain intrinsically disordered regions, 17 **septal pore-associated (SPA) proteins** were identified in *N. crassa* (Lai et al. 2012). Intrinsically disordered proteins range from partially to completely unstructured and lacking globular folds; however, these proteins are capable of folding upon binding to target molecules (Wright and Dyson 2009). The number of reports related to intrinsically disordered proteins has increased significantly in recent years, and many biological functions of these proteins have been revealed (Oldfield and Dunker 2014). The loss-of-function of several SPA proteins leads to excessive septation, septal pore degeneration, and uncontrolled Woronin body activation (Lai et al. 2012). Spa10 protein is required for the localization of LAH C-terminal region at the mature septal pore, consequently stable tethering of Woronin bodies to the septum (Leonhardt et al. 2017). These findings suggest that the septal pore is a complex subcellular site for the assembly of unstructured proteins, which contribute to diverse states of cell-to-cell connectivity.

The *Pezizomycotina*-specific protein SO, which was originally found in *N. crassa* and was named based on the "soft" appearance of the mutant colony, is important for hyphal fusion and sexual reproduction (Fleißner et al. 2005; Engh et al. 2007). SO protein consists of approximately 1200 amino acids and contains a single WW domain. Pro40, a *Sordaria macrospora* SO homolog, functions as a scaffold by associating with protein kinases involved in cell wall integrity (Teichert et al. 2014). SO protein is dispersed throughout the cytoplasm under normal growth conditions but accumulates at
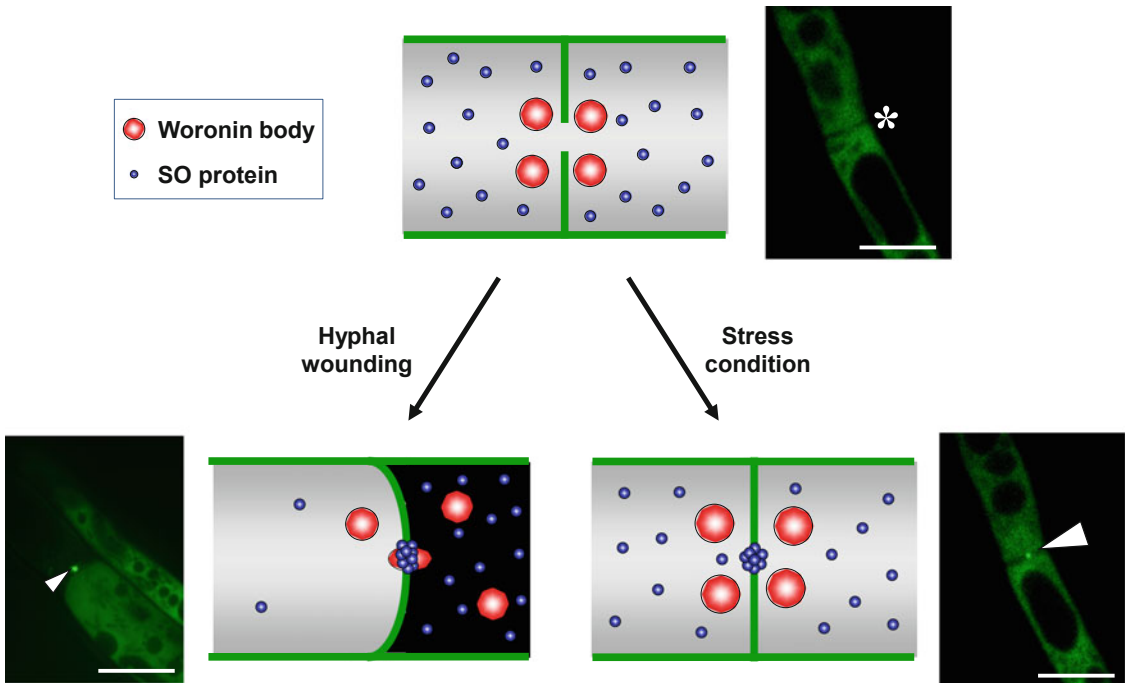
Fig. 6 Accumulation of SO protein at the septal pore. SO localization was visualized by expressing as EGFP fusion protein. (Left) Upon hyphal wounding, SO accumulates at the septal pore together with the Woronin body. (Right) SO also accumulates at the septal pore under stressed conditions. Asterisk indicates the septum, and the arrowhead indicates the accumulation of SO protein at the septal pore. Bars: 5 μm

the septal pore adjacent to wounded cells (Fig. 6), as was reported in *N. crassa* and *A. oryzae* (Fleißner and Glass 2007; Maruyama et al. 2010). *S. macrospora* Pro40 protein colocalizes with Woronin bodies (Engh et al. 2007). Deletion of the *so* gene delays septal plugging and reduces the number of hyphae that prevent excessive cytoplasmic loss in *N. crassa* and *A. oryzae* (Fleißner and Glass 2007; Maruyama et al. 2010). Taken together, these findings indicate that SO protein has septal plugging activity by accumulating at the septal pore, similar to the function of Woronin bodies. Additionally, SO protein accumulates at the septal pore in aging hyphae (Fleißner and Glass 2007) and under various stress conditions, including low/high temperature, high acidity/alkalinity, and nitrogen/carbon depletion (Fig. 6) (Maruyama et al. 2010). In response to pulse laser treatment, which physically stresses cells without causing hyphal wounding, SO rapidly accumulates at the septal pore nearest to the stressed hyphal area (Maruyama et al. 2010).

Thus, SO protein may regulate cell-to-cell connectivity via the septal pore in a stress-dependent manner.

A study of *Aspergillus nidulans* revealed that mitosis interrupts cell-to-cell connectivity through the septal pore (Shen et al. 2014). The mitotic NIMA kinase was found to be localized to the septum during interphase and to contribute to keeping the septal pore open but is translocated into the nucleus to initiate mitosis, resulting in septal closure. Notably, however, the mitotic regulation of cell-to-cell connectivity is independent of Woronin bodies and SO protein. Identification of the NIMA kinase substrate(s) may provide insight into the molecular mechanisms underlying mitotic interruption of cell-to-cell connectivity.

VII. Conclusions and Perspectives

Despite the discovery of Woronin bodies over 150 years ago, our understanding of these unique fungal organelles was exclusively based on primitive microscopic studies conducted in

the twentieth century. However, in the past 20 years, molecular structure and function of Woronin bodies have been largely elucidated. Genomic changes such as gene duplication and splitting generated and modified the Woronin body to allow for specific multicellular organization of individual *Pezizomycotina* species. Although the primary function of Woronin bodies had long been thought to be wound healing, recent molecular studies have revealed new physiological functions of these organelles. To date, however, the molecular machineries controlling and mediating septal closure and cell repair remain mostly unknown. Further investigations to functionally link Woronin bodies and related molecules in the vicinity of the septum will lead to a more comprehensive understanding of the mechanisms regulating cell-to-cell connectivity and fungal multicellularity.

References

Asiegbu FO, Choi W, Jeong JS, Dean RA (2004) Cloning, sequencing and functional analysis of *Magnaporthe grisea MVP1* gene, a *hex-1* homolog encoding a putative "Woronin body" protein. FEMS Microbiol Lett 230:85–90

Beck J, Ebel F (2013) Characterization of the major Woronin body protein HexA of the human pathogenic mold *Aspergillus fumigatus*. Int J Med Microbiol 303:90–97

Beck J, Echtenacher B, Ebel F (2013) Woronin bodies, their impact on stress resistance and virulence of the pathogenic mould *Aspergillus fumigatus* and their anchoring at the septal pore of filamentous Ascomycota. Mol Microbiol 89:857–871

Berns MW, Aist JR, Wright WH, Liang H (1992) Optical trapping in animal and fungal cells using a tunable, near-infrared titanium-sapphire laser. Exp Cell Res 198:375–378

Bleichrodt RJ, van Veluw GJ, Recter B, Maruyama J, Kitamoto K, Wösten HA (2012) Hyphal heterogeneity in *Aspergillus oryzae* is the result of dynamic closure of septa by Woronin bodies. Mol Microbiol 86:1334–1344

Bleichrodt RJ, Hulsman M, Wösten HA, Reinders MJ (2015) Switching from a unicellular to multicellular organization in an *Aspergillus niger* hypha. MBio 6:e00111

Bowman JC, Scott Hicks P, Kurtz MB, Rosen H, Schmatz DM, Liberator PA, Douglas CM (2002) The antifungal echinocandin caspofungin acetate kills growing cells of *Aspergillus fumigatus in vitro*. Antimicrob Agents Chemother 46:3001–3012

Buller AHR (1933) The translocation of protoplasm through septate mycelium of certain Pyrenomycetes, Discomycetes and Hymenomycetes. In: Researches on fungi, vol 5. Longmans, Green & Co, London, pp 75–167

Camp RR (1977) Association of microbodies, Woronin bodies, and septa in intercellular hyphae of *Cymadothea trifolii*. Can J Bot 55:1856–1859

Curach NC, Te'o VS, Gibbs MD, Bergquist PL, Nevalainen KM (2004) Isolation, characterization and expression of the *hex1* gene from *Trichoderma reesei*. Gene 331:133–140

Edgerton-Morgan H, Oakley BR (2012) gamma-Tubulin plays a key role in inactivating APC/C(Cdh1) at the G(1)-S boundary. J Cell Biol 198:785–791

Engh I, Würtz C, Witzel-Schlömp K, Zhang HY, Hoff B, Nowrousian M, Rottensteiner H, Kück U (2007) The WW domain protein PRO40 is required for fungal fertility and associates with Woronin bodies. Eukaryot Cell 6:831–843

Escaño CS, Juvvadi PR, Jin FJ, Takahashi T, Koyama Y, Yamashita S, Maruyama J, Kitamoto K (2009) Disruption of the *Aopex11-1* gene involved in peroxisome proliferation leads to impaired Woronin body formation in *Aspergillus oryzae*. Eukaryot Cell 8:296–305

Fiddy C, Trinci AP (1976) Mitosis, septation, branching and the duplication cycle in *Aspergillus nidulans*. J Gen Microbiol 97:169–184

Fleißner A, Glass NL (2007) SO, a protein involved in hyphal fusion in *Neurospora crassa*, localizes to septal plugs. Eukaryot Cell 6:84–94

Fleißner A, Sarkar S, Jacobson DJ, Roca MG, Read ND, Glass NL (2005) The *so* locus is required for vegetative cell fusion and postfertilization events in *Neurospora crassa*. Eukaryot Cell 4:920–930

Han P, Jin FJ, Maruyama J, Kitamoto K (2014) A large nonconserved region of the tethering protein Leashin is involved in regulating the position, movement, and function of Woronin bodies in *Aspergillus oryzae*. Eukaryot Cell 13:866–877

Jedd G (2011) Fungal evo-devo: organelles and multicellular complexity. Trends Cell Biol 21:12–19

Jedd G, Chua NH (2000) A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. Nat Cell Biol 2:226–231

Jedd G, Pieuchot L (2012) Multiple modes for gatekeeping at fungal cell-to-cell channels. Mol Microbiol 86:1291–1294

Juvvadi PR, Maruyama J, Kitamoto K (2007) Phosphorylation of the *Aspergillus oryzae* Woronin body protein, AoHex1, by protein kinase C: evidence for its role in the multimerization and proper localization of the Woronin body protein. Biochem J 405:533–540

Keller GA, Krisans S, Gould SJ, Sommer JM, Wang CC, Schliebs W, Kunau W, Brody S, Subramani S (1991) Evolutionary conservation of a microbody targeting signal that targets proteins to peroxisomes, glyoxysomes, and glycosomes. J Cell Biol 114:893–904

Kim KK, Hung LW, Yokota H, Kim R, Kim SH (1998) Crystal structures of eukaryotic translation initiation factor 5A from *Methanococcus jannaschii* at 1.8 A resolution. Proc Natl Acad Sci USA 95:10419–10424

Kim KH, Willger SD, Park SW, Puttikamonkul S, Grahl N, Cho Y, Mukhopadhyay B, Cramer RA Jr, Lawrence CB (2009) TmpL, a transmembrane protein required for intracellular redox homeostasis and virulence in a plant and an animal fungal pathogen. PLoS Pathog 5:e1000653

Kubo Y, Fujihara N, Harata K, Neumann U, Robin GP, O'Connell R (2015) *Colletotrichum orbiculare FAM1* encodes a novel Woronin body-associated Pex22 peroxin required for appressorium-mediated plant infection. MBio 6:e01305–e01315

Lai J, Koh CH, Tjota M, Pieuchot L, Raman V, Chandrababu KB, Yang D, Wong L, Jedd G (2012) Intrinsically disordered proteins aggregate at fungal cell-to-cell channels and regulate intercellular connectivity. Proc Natl Acad Sci USA 109:15781–15786

Leonhardt Y, Kakoschke SC, Wagener J, Ebel F (2017) Lah is a transmembrane protein and requires Spa10 for stable positioning of Woronin bodies at the septal pore of *Aspergillus fumigatus*. Sci Rep 7:44179

Lew RR (2005) Mass flow and pressure-driven hyphal extension in *Neurospora crassa*. Microbiology 151:2685–2692

Li H, Oberhauser AF, Redick SD, Carrion-Vazquez M, Erickson HP, Fernandez JM (2001) Multiple conformations of PEVK proteins detected by single-molecule techniques. Proc Natl Acad Sci USA 98:10682–10686

Li L, Wang J, Zhang Z, Wang Y, Liu M, Jiang H, Chai R, Mao X, Qiu H, Liu F, Sun G (2014) MoPex19, which is essential for maintenance of peroxisomal structure and Woronin bodies, is required for metabolism and development in the rice blast fungus. PLoS One 9:e85252

Lichius A, Yáñez-Gutiérrez ME, Read ND, Castro-Longoria E (2012) Comparative live-cell imaging analyses of SPA-2, BUD-6 and BNI-1 in *Neurospora crassa* reveal novel features of the filamentous fungal polarisome. PLoS One 7:e30372

Liu F, Ng SK, Lu Y, Low W, Lai J, Jedd G (2008) Making two organelles from one: Woronin body biogenesis by peroxisomal protein sorting. J Cell Biol 180:325–339

Liu F, Lu Y, Pieuchot L, Dhavale T, Jedd G (2011) Import oligomers induce positive feedback to promote peroxisome differentiation and control organelle abundance. Dev Cell 21:457–468

Managadze D, Würtz C, Sichting M, Niehaus G, Veenhuis M, Rottensteiner H (2007) The peroxin PEX14 of *Neurospora crassa* is essential for the biogenesis of both glyoxysomes and Woronin bodies. Traffic 8:687–701

Markham P (1994) Occlusions of septal pores in filamentous fungi. Mycol Res 98:1089–1106

Markham P, Collinge AJ (1987) Woronin bodies of filamentous fungi. FEMS Microbiol Rev 46:1–11

Maruyama J, Kitamoto K (2007) Differential distribution of the endoplasmic reticulum network in filamentous fungi. FEMS Microbiol Lett 272:1–7

Maruyama J, Juvvadi PR, Ishi K, Kitamoto K (2005) Three-dimensional image analysis of plugging at the septal pore by Woronin body during hypotonic shock inducing hyphal tip bursting in the filamentous fungus *Aspergillus oryzae*. Biochem Biophys Res Commun 331:1081–1088

Maruyama J, Kikuchi S, Kitamoto K (2006) Differential distribution of the endoplasmic reticulum network as visualized by the BipA-EGFP fusion protein in hyphal compartments across the septum of the filamentous fungus, *Aspergillus oryzae*. Fungal Genet Biol 43:642–654

Maruyama J, Escaño CS, Kitamoto K (2010) AoSO protein accumulates at the septal pore in response to various stresses in the filamentous fungus *Aspergillus oryzae*. Biochem Biophys Res Commun 391:868–873

Mason PJ, Crosse R (1975) Crystalline inclusions in hyphae of the glaucus group of Aspergilli. Trans Br Mycol Soc 65:129–134

McKeen WE (1971) Woronin bodies in *Erysiphe graminis* DC. Can J Microbiol 17:1557–1560

Momany M, Richardson EA, Van Sickle C, Jedd G (2002) Mapping Woronin body position in *Aspergillus nidulans*. Mycologia 94:260–266

Müller WH, Montijn RC, Humbel BM, van Aelst AC, Boon EJ, van der Krift TP, Boekhout T (1998) Structural differences between two types of basidiomycete septal pore caps. Microbiology 144:1721–1730

Nave R, Fürst DO, Weber K (1989) Visualization of the polarity of isolated titin molecules: a single globular head on a long thin rod as the M band anchoring domain? J Cell Biol 109:2177–2187

Nayak T, Edgerton-Morgan H, Horio T, Xiong Y, De Souza CP, Osmani SA, Oakley BR (2010) Gamma-tubulin regulates the anaphase-promoting complex/cyclosome during interphase. J Cell Biol 190:317–330

Ng SK, Liu F, Lai J, Low W, Jedd G (2009) A tether for Woronin body inheritance is associated with evolutionary variation in organelle positioning. PLoS Genet 5:e1000521

Oldfield CJ, Dunker AK (2014) Intrinsically disordered proteins and intrinsically disordered protein regions. Annu Rev Biochem 83:553–584

Peat TS, Newman J, Waldo GS, Berendzen J, Terwilliger TC (1998) Structure of translation initiation factor 5A from *Pyrobaculum aerophilum* at 1.75 A resolution. Structure 6:1207–1214

Plamann M (2009) Cytoplasmic streaming in *Neurospora*: disperse the plug to increase the flow? PLoS Genet 5:e1000526

Ryan FJ, Beadle GW, Tatum EL (1943) The tube method of measuring the growth rate of Neurospora. Am J Bot 30:784–799

Shen KF, Osmani AH, Govindaraghavan M, Osmani SA (2014) Mitotic regulation of fungal cell-to-cell connectivity through septal pores involves the NIMA kinase. Mol Biol Cell 25:763–775

Son M, Lee KM, Yu J, Kang M, Park JM, Kwon SJ, Kim KH (2013) The HEX1 gene of *Fusarium graminearum* is required for fungal asexual reproduction and pathogenesis and for efficient viral RNA accumulation of *Fusarium graminearum* virus 1. J Virol 87:10356–10367

Soundararajan S, Jedd G, Li X, Ramos-Pamploña M, Chua NH, Naqvi NI (2004) Woronin body function in *Magnaporthe grisea* is essential for efficient pathogenesis and for survival during nitrogen starvation stress. Plant Cell 6:1564–1574

Steinberg G, Schuster M, Hacker C, Kilaru S, Correia A (2017a) ATP prevents Woronin bodies from sealing septal pores in unwounded cells of the fungus *Zymoseptoria tritici*. Cell Microbiol 19:e12764

Steinberg G, Harmer NJ, Schuster M, Kilaru S (2017b) Woronin body-based sealing of septal pores. Fungal Genet Biol 109:53–55

Tegelaar M, Wösten HAB (2017) Functional distinction of hyphal compartments. Sci Rep 7:6039

Teichert I, Steffens EK, Schnaß N, Fränzel B, Krisp C, Wolters DA, Kück U (2014) PRO40 is a scaffold protein of the cell wall integrity pathway, linking the MAP kinase module to the upstream activator protein kinase C. PLoS Genet 10:e1004582

Tenney K, Hunt I, Sweigard J, Pounder JI, McClain C, Bowman EJ, Bowman BJ (2000) Hex-1, a gene unique to filamentous fungi, encodes the major protein of the Woronin body and functions as a plug for septal pores. Fungal Genet Biol 31:205–217

Tey WK, North AJ, Reyes JL, Lu YF, Jedd G (2005) Polarized gene expression determines Woronin body formation at the leading edge of the fungal colony. Mol Biol Cell 16:2651–2659

Trinci APJ, Collinge AJ (1974) Occlusion of the septal pores of damaged hyphae of *Neurospora crassa* by hexagonal crystals. Protoplasma 80:57–67

Wergin WP (1973) Development of Woronin bodies from microbodies in *Fusarium oxysporum* f. sp. *lycopersici*. Protoplasma 76:249–260

Woronin M (1864) Zur Entwicklungsgeschichte der Ascobolus pulcherrimus Cr und eigiger Pezizen. Abh Senkenb Naturforsch Ges 5:333–344

Wright PE, Dyson HJ (2009) Linking folding and binding. Curr Opin Struct Biol 19:31–38

Würtz C, Schliebs W, Erdmann R, Rottensteiner H (2008) Dynamin-like protein-dependent formation of Woronin bodies in *Saccharomyces cerevisiae* upon heterologous expression of a single protein. FEBS J 275:2932–2941

Yuan P, Jedd G, Kumaran D, Swaminathan S, Shio H, Hewitt D, Chua NH, Swaminathan K (2003) A HEX-1 crystal lattice required for Woronin body function in *Neurospora crassa*. Nat Struct Biol (4):264–270

Zekert N, Veith D, Fischer R (2010) Interaction of the *Aspergillus nidulans* microtubule-organizing center (MTOC) component ApsB with gamma-tubulin and evidence for a role of a subclass of peroxisomes in the formation of septal MTOCs. Eukaryot Cell 9:795–805