

Molecular investigation of Woronin body function and its application in *Aspergillus oryzae*

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Research aims

Species of Pezizomycotina (filamentous ascomycetes) grow via elongation of the hyphal tip to form straight primary hyphae with branches. The hyphae are divided into distinct cells by the formation of septa; thus, filamentous fungi are characterized by multicellularity. Cytoplasmic continuity between adjacent cells through the septal pore is associated with the risk of cytoplasmic loss in adjacent cells upon hyphal lysis. This risk was clearly demonstrated by the exposure of the industrial filamentous fungus *Aspergillus oryzae* grown on agar medium to hypotonic shock, which caused most of the hyphal tips to burst and lose their cytoplasmic constituents (Fig. 1A).¹⁾ However, ~80% of the cells immediately adjacent to the lysed tip cells retained their cytoplasmic constituents,¹⁾ allowing them to initiate regrowth by creating a new hyphal tip.²⁾

The Woronin body is a unique organelle present in Pezizomycotina species that plugs the septal pore upon hyphal lysis and prevents excessive cytoplasmic loss from the cell adjacent to the lysed cell (Fig. 1B). Previously, we characterized AoHex1 as the major protein of Woronin body in *A. oryzae*, and investigated Woronin body differentiation from the peroxisome.^{1,3)}

However, how Woronin bodies localize to the vicinity of the septum is not known. Recently, the Leashin (LAH) protein was found to tether Woronin bodies to the cell cortex in *Neurospora crassa*,⁴⁾ which surprisingly does not recruit the organelle to the vicinity of the septum. In this study, we attempted to elucidate the mechanism for tethering Woronin bodies at the septum in *A. oryzae*.

Methods

Gene disruption in *A. oryzae*

Gene disruption in *A. oryzae* strains was done by replacement of coding sequences with the *adeA* marker gene, and was confirmed by Southern blot analysis.

Transmission electron microscopic analysis

Mycelia grown on DPY agar medium at 30°C for 4 days were fixed with glutaraldehyde and osmium tetroxide. Samples were observed by transmission electron microscopy (Model JEM-1010; JEOL, Japan).

Hypotonic shock experiment

To induce hyphal tip bursting, 1 ml of water was added to *A. oryzae* strains cultured on a thin layer of DPY agar medium at 30°C for 24 h and observed by differential interference contrast microscopy as described in Maruyama *et al.*¹⁾

Results

Functional analysis of AoLAH protein

We identified one gene (AO090011000895) encoding a protein showing similarity to *N. crassa* LAH in the *A. oryzae* genome database. According to homology comparison, the ORF region of the gene was corrected. We subsequently named this gene *Aolah* (*A. oryzae* *lah*). The *Aolah* gene was predicted to encode a single polypeptide of 5,727 amino acids, which is homologous to two *N. crassa* LAH proteins (LAH-1 and LAH-2) expressed from the unidirectionally aligned locus.

In order to examine the function of AoLAH, we disrupted the *Aolah* gene. Transmission electron microscopic

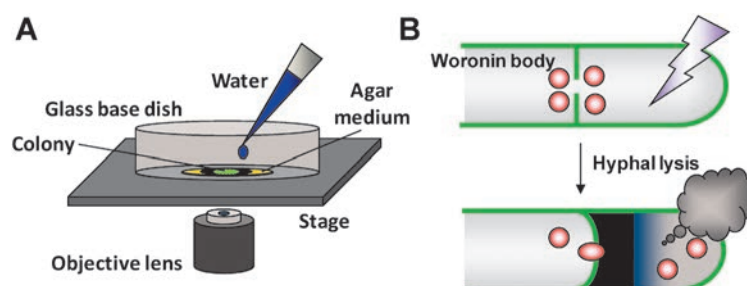


Fig. 1. Hypotonic shock experiment to induce hyphal tip bursting (A) and septal pore plugging by the Woronin body (B).

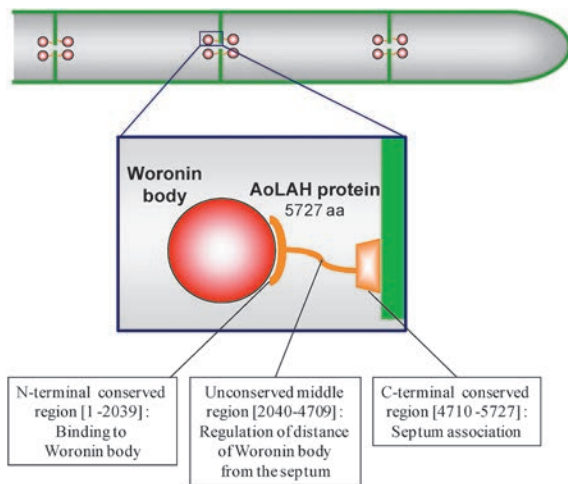


Fig. 2. Structure and function of the AoLAH protein.

analysis showed that, in the wild-type strain, Woronin bodies appeared as electron-dense spherical structures close to the septum. However, in the *Aolah* disruptant, no tethered Woronin bodies were observed in the vicinity of the septum. There were un-tethered Woronin bodies along the hyphae. It was therefore concluded that AoLAH is required for Woronin body tethering at the septum in *A. oryzae*.

To investigate the role of AoLAH in Woronin body function, we performed a hypotonic shock experiment to induce hyphal tip bursting (Fig. 1A). In contrast to the wild-type strain that exhibited an approximately 80% ability to retain cytoplasmic constituents in the second cell, the *Aolah* disruptant showed a reduced ability with approximately 60%. This indicates the functional involvement of AoLAH in the septal plugging ability of the Woronin body.

Deletion analysis of AoLAH protein

AoLAH was predicted to be a very large protein with over 5,000 amino acids. To analyze the functional role of individual regions, we analyzed the localization of N-terminal and C-terminal regions by expressing each individually as EGFP fusion proteins in *A. oryzae*.

When the N-terminal conserved region of AoLAH (AoLAH[1–2039]) was expressed as an EGFP fusion protein, it was detected on both sides of the septum at a defined distance from the septal pore, which is the localization pattern typical for Woronin bodies in the vicinity of the septum. Upon hyphal lysis by hypotonic shock, AoLAH[1–2039]-EGFP moved to the septal pore similar to Woronin bodies. In the *Aolah*-disrupted background, there was no

fluorescence observed at the septal pore, which is consistent with the transmission electron microscopic analysis. These data revealed a role for the AoLAH N-terminal region in the association with Woronin bodies.

The C-terminal conserved region of AoLAH (AoLAH[4710–5727]) expressed as an EGFP fusion protein was detected close to the septal pore in both the wild-type strain and the *Aolah* disruptant, indicating that the AoLAH C-terminal region itself is associated with the septum.

When a fusion of the AoLAH N- and C-terminal regions lacking the non-conserved middle region was expressed in the *Aolah* disruptant, Woronin bodies were located in the vicinity of the septum, although they were closer to the septum. This suggests that the non-conserved middle region of AoLAH has a role in regulating the distance of Woronin bodies from the septum.

Conclusion

Recently, we reported that the Woronin body reversibly closes the septal pore during normal growth of *A. oryzae*, a function that impedes cytoplasmic continuity between adjacent cells during normal growth and helps maintain hyphal heterogeneity in mycelia.⁵⁾ Although wild-type cells show a heterogeneous distribution of gene expression activity, the absence of Woronin bodies results in uniform activity in different cells. Based on our results, investigation of the role of AoLAH in plugging the septal pore during normal growth is warranted.

References

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