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AoSO protein accumulates at the septal pore in response to various stresses in the filamentous fungus *Aspergillus oryzae*

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ABSTRACT

Filamentous ascomycetes form hyphal networks that are compartmentalized by septa which have a perforated pore allowing the passage of cytoplasm and organelles between adjacent hyphal compartments. Thus, the septal pore may play an important role in the organized growth of multicellular organisms. Upon hyphal injury, the septal pore is plugged by a wound-healing organelle, known as the Woronin body, to prevent excessive cytoplasmic leakage. However, the movement of proteins towards the septal pore in response to stress has not been extensively studied in filamentous fungi. In this study, we identified an *Aspergillus oryzae* protein, AoSO, which is homologous to the *Neurospora crassa* SO protein that was reported to accumulate at the septal pore in aging hyphae. The Δ*Aoso* strain showed excessive cytoplasmic leakage upon hyphal injury similar to the Woronin body-deficient strain Δ*Aohex1*. Cellular localization studies using EGFP showed that AoSO accumulated at the septal pore adjacent to the injured compartment, while it was dispersed throughout the cytoplasm under normal growth conditions. These results indicate that AoSO plays a role in preventing excessive cytoplasmic leakage upon hyphal injury by accumulating at the septal pore. Furthermore, AoSO accumulated at the septal pore in response to various stresses, including low and high temperature, extreme acidic and alkaline pH, and nitrogen and carbon depletion. Physical stress induced by pulse laser treatment on a hyphal region at a distance from the septum caused accumulation of the AoSO protein at the septal pore within only a few minutes. This study presents a novel behavior in which a filamentous fungal protein relocalizes to the septal pore in response to various stresses.

Introduction

Filamentous ascomycetes grow at their apices to form straight hyphae and subapical branches which are compartmentalized by septa. The presence of septum divides the hyphae into distinct cells which classifies filamentous fungi as multicellular organisms. The possible functions of the septum include increasing the structural integrity of the hyphae and division of the mycelium into distinct sections for developmental processes such as sexual and asexual reproduction. However, the septum does not completely separate the hyphae due to the presence of a septal pore which allows the passage of cytoplasm and organelles between adjacent hyphal compartments [1–6]. This characteristic is shared in higher eukaryotic organisms as gap junctions in animal cells and plasmodesmata in plant cells.

Cytoplasmic continuity with adjacent compartments would cause catastrophic risks when hyphae are damaged if it were not for the Woronin body, a unique organelle in filamentous ascomycetes which plugs the septal pore and prevents excessive cytoplasmic leakage from the hyphae [7]. This was demonstrated by deletion of the *hex1* gene, encoding a Woronin body protein, which resulted in excessive loss of the cytoplasm upon hyphal injury [8,9]. Using fluorescence microscopy, an *Aspergillus oryzae* protein characteristic of a Woronin body, known as AoHex1, was found to plug the septal pore adjacent to an injured hyphal compartment [9].

Previous electron microscopic analysis has shown that septal pores in aging hyphae are plugged by electron-dense material distinct from Woronin bodies [10]. Aging hyphae encounter a variety of environmental stresses, such as nutrient depletion, which often suddenly occur during vegetative growth. To maintain homeostasis during vegetative growth as multicellular organisms, filamentous fungi require an acute system for the regulation of cytoplasmic continuity between adjacent compartments in response to these sudden environmental changes. It is hypothesized that there are proteins capable of relocalizing to the septal pore in response to stress, however, such proteins have not been extensively studied in filamentous fungi.

Among several proteins known to localize at the septal pore in filamentous ascomycetes, *Neurospora crassa* SO protein and its homolog in *Sordaria macrospora*, Pro40, exhibit an interesting localization behavior. Under normal growth conditions, they do

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not localize at the septal pore, but are found at septal pores adjacent to injured compartments [11,12], a characteristic which is similar to the Woronin body. The SO and Pro40 proteins were identified to be necessary for hyphal fusion [13] and sexual reproduction [12], respectively. The SO protein was also reported to accumulate at the septal pore in aging hyphae [11] which raises the possibility that it may be capable of responding to a variety of environmental stresses.

In the present study, we identified and disrupted the *A. oryzae Aoso* gene, a homolog of the *N. crassa so* gene, and studied its role in the prevention of excessive cytoplasmic leakage upon hyphal injury. We also constructed the fusion protein AoSO-EGFP in an attempt to visualize the subcellular localization of AoSO in response to various stresses.

Materials and methods

Strains and growth media. The *A. oryzae* wild type-strain RIB40 was used as a DNA donor. *Escherichia coli* DH5α was used for DNA manipulation. *A. oryzae* NSRKu70-1-1 (*niaD*$^-$ *sC*$^-$ *adeA*$^-$ Δ*argB::adeA*$^-$ Δ*ku70::argB*) [14] was used as host strain for *Aoso* gene disruption. DPY medium [14] was used for liquid cultivation and growth analyses of the *A. oryzae* strains. Czapek Dox (CD) + Met (2% glucose, 0.3% $NaNO_3$, 0.2% KCl, 0.1% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.002% $FeSO_4{\cdot}7H_2O$ and 0.0015% methionine, pH 5.5) and M + Met medium [14] were used for transformation and growth analyses of *A. oryzae*. Transformation of *A. oryzae* was carried out as described previously by Kitamoto [15]. For the counting of conidia, mycelia were first streaked on Potato Dextrose (PD) medium and incubated at 30 °C for 5 days. Plates were flooded with 10 ml 0.01% Tween-80 and the conidia were then harvested for counting.

Construction of the ΔAoso strain. For disruption of the *Aoso* gene, the plasmid pgdAoSO was constructed using the MultiSite Gateway™ cloning system (Invitrogen, Carlsbad, CA, USA) [16]. The 1.5 kb upstream region of the *Aoso* gene was amplified by PCR using the primers aB4F_usAoSO (5′-GGGGACAACTTTGTATAGAAAAGTTGCAAGATGGTTTTAGGAAGCGACCTGC-3′) and aB1R_usAoSO (5′-GGGGACTGCTTTTTTGTACAAACTTGGAGGTAATGAGGGGG ATCT TTAGGAG-3′), while the 1.5 kb downstream region of the gene was amplified using aB2F_dsAoSO (5′-GGGGACAGCTTTCTTGTACAAAGTGGCTTCGGGTCTGCATCCCTTTATGCTT-3′) and aB3R_dsAoSO (5′-GGGGACAACTTTGTATAATAAAGTTGGCTTTCCGTGGCACCTATTC AATCAC-3′). The underlined sequences represent MultiSite Gateway *att*B recombination sites. The upstream and downstream DNA fragments of the *Aoso* gene were cloned by the BP clonase reaction into the pDONR-P4-P1R and pDONR-P2R-P3 entry vectors, respectively. The obtained 5′ and 3′ entry clones and the center entry clone, pgEaA (containing the *adeA* marker gene) [17], were subjected to the LR clonase reaction in the presence of pDEST R4-R3 (destination vector) to obtain the plasmid pgdAoSO. The deletion fragment for the *Aoso* gene was amplified by PCR using the plasmid pgdAoSO as a template and the primers aB4F_usAoSO and aB3R_dsAoSO. The deletion fragment was introduced into the NSRKu70-1-1 strain and M + Met medium was used for selection of *adeA*$^+$ transformants.

Disruption of the *Aoso* gene was confirmed by Southern blotting. Briefly, after electrophoresis, digested genomic DNA was transferred onto Hybond N + membrane (GE Healthcare, Buckinghamshire, UK). The ECL (enhanced chemiluminescence) direct nucleic acid labeling and detection system (GE Healthcare, Buckinghamshire, UK) and a LAS-1000plus luminescent image analyzer (Fuji Photo Film, Tokyo, Japan) were used for detection.

All six Δ*Aoso* strains obtained in this study showed nearly identical phenotypes. The NSK-ΔSO11 strain (*niaD*$^-$ *sC*$^-$ *adeA*$^-$ Δ*argB::adeA*$^-$ Δ*ku70::argB* Δ*Aoso::adeA*) was used as the representative Δ*Aoso* strain. The NSRKu70-1-1A strain (*niaD*$^-$ *sC*$^-$ *adeA*$^-$ Δ*argB::adeA*$^-$ Δ*ku70::argB adeA*), a transformant with a plasmid containing the *adeA* maker gene from the NSRKu70-1-1 strain [14], was used as the control strain.

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 DIC (differential interference contrast) microscopy as described in Maruyama et al. [9].

Complementation of the ΔAoso strain. The plasmid pgPaBSG was constructed to express the AoSO-EGFP fusion protein under the control of the *amyB* promoter by using the MultiSite Gateway™ cloning system. The *Aoso* gene open reading frame was amplified by PCR using the primers attB1_SO-F2 (5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTtaATGAATCCCAGCAAGGCCCGTCA-3′) and attB2_SO-R2 (5′-GGGGACCACTTTGTACAAGAAAGCTGGGTATTTCCCATGTCCTAAG CTTGGGATAATG). The underlined sequences represent MultiSite Gateway *att*B recombination sites. The amplified *att*B-flanked *Aoso* gene was introduced into the entry vector pDONR 221 by the BP clonase reaction. The resultant center entry clone was named pgESO. The 5′ entry clone pgPaB (*amyB* promoter; [16]), center entry clone pgESO, 3′ entry clone pg3′E (*egfp*; [16]), and the destination vector pgDN containing *A. oryzae niaD* as a selectable marker [16] were subjected to the LR clonase reaction to generate the plasmid pgPaBSG. This plasmid was then introduced into the Δ*Aoso* strain NSK-ΔSO11 and CD + Met medium was used for the selection of *niaD*$^+$ transformants. The generated strain was named NSK-ASG1 (*niaD*$^-$ *sC*$^-$ *adeA*$^-$ Δ*argB::adeA*$^-$ Δ*ku70::argB* Δ*Aoso::adeA* P-*amyB::Aoso-egfp::niaD*). Plasmid pNR10 (containing *niaD* as a selectable marker) was introduced into NSK-ΔSO11, generating strain NSK-ΔSON9 (*niaD*$^-$ *sC*$^-$ *adeA*$^-$ Δ*argB::adeA*$^-$ Δ*ku70::argB* Δ*Aoso::adeA niaD*) which served as the negative control of the complementation. NSRKu70-1-1AN (*niaD*$^-$ *sC*$^-$ *adeA*$^-$ Δ*argB::adeA*$^-$ Δ*ku70::argB adeA niaD*) [14] was used as a control.

Confocal microscopy. Conidia (1×10^3) of the NSK-ASG1 strain were pre-inoculated into 100 μl CD + Met liquid medium on a glass bottom dish, and incubated at 30 °C for 18 h before being subjected to various stresses. Glucose was used as a carbon source, with which the *amyB* promoter is activated at an intermediate level of expression [18]. The stresses used were low (4 °C) or high (45 °C) temperature, low (2) or high (10) pH, and either carbon (glucose and methionine) or nitrogen (nitrate and methionine) source depletion. For physical stress, pulse laser treatment with a power intensity of level 6 directed at 4 spots in close proximity with 5 pulses for each spot in a hyphal region 10–15 μm from the septum was made using the Micropoint Laser Illumination System (Photonic Instruments, Inc., Saint Charles, IL, USA). Hyphal injury, such as cytoplasmic bleeding, was not observed under these conditions. The cultures were observed by confocal laser scanning microscopy using an IX71 inverted microscope (Olympus, Tokyo, Japan) equipped with a 100× Neofluor objective lens (1.40 numerical aperture), 488 nm semiconductor laser (Furukawa Electric, Tokyo, Japan), GFP filters (Nippon Roper, Chiba, Japan), a CSU22 confocal scanning system (Yokogawa Electronics, Tokyo, Japan), and an Andor iXon cooled digital CCD camera (Andor Technology PLC, Belfast, UK). Images were analyzed with Andor iQ software (Andor Technology PLC) and Metamorph software (Molecular Devices, Sunnyvale, CA, USA).

Results

A. oryzae possesses a gene homologous to the so gene

Although the *so* gene is conserved in the genomes of filamentous ascomycetes, homologs are not found in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, or basidiomycete species [13]. The *A. oryzae so* gene homolog was identified in the *A. oryzae* gen-

ome database (DOGAN; http://www.bio.nite.go.jp/dogan/Top) and designated as *Aoso* (AO090003000023). cDNA cloning confirmed that the *Aoso* gene contains two introns and three exons and encodes a polypeptide of 1195 amino acids. AoSO showed 48% identity with both the *N. crassa* SO and *S. macrospora* Pro40 proteins, and had a higher identity with other Aspergilli, including *A. fumigatus* (Afu6g06520) (74%) and *A. nidulans* (ANID_05776.1) (70%).

Disruption of the Aoso gene and phenotypic analysis of the ΔAoso strain

In order to examine the function of AoSO, we first generated an Δ*Aoso* strain in *A. oryzae* by replacement of the *Aoso* gene with the *adeA* marker gene, and confirmed the gene disruption by Southern analysis (data not shown). To determine if disruption of the *Aoso* gene resulted in any growth defects, the Δ*Aoso* strain was examined on agar media. While there were no significant variations in the growth of the Δ*Aoso* strain on nutrient-rich medium, it exhibited slower growth on minimal medium as compared with the control strain (Fig. 1). The Δ*Aoso* strain also produced fewer conidia (Fig. 2A), which is similar to the result observed in the *N. crassa so* mutant [13].

We next investigated whether the lack of AoSO resulted in excessive cytoplasmic leakage upon hyphal injury by the addition of water to the Δ*Aoso* strain grown on agar medium to induce hypotonic shock and hyphal tip bursting [9,14]. It was previously shown that the absence of Woronin bodies in the Δ*Aohex1* strain resulted in excessive cytoplasmic leakage from the second compartment adjacent to the burst apical compartment [9]. After inducing hypotonic shock in the Δ*Aoso* strain, the ability to retain cytoplasmic constituents in the second compartment adjacent to the injured compartment was examined by DIC microscopic observation. In contrast to the control strain in which the cytoplasmic constituents were retained in 85% of the second compartments in the damaged hyphae, the Δ*Aoso* strain retained only 50% of this ability (Fig. 2B).

Complementation of the ΔAoso strain with the AoSO-EGFP fusion protein

To determine whether the AoSO-EGFP fusion protein was functional in *A. oryzae*, it was used to complement the Δ*Aoso* strain. When the plasmid for expression of the AoSO-EGFP fusion protein was introduced into the Δ*Aoso* strain, the transformants showed almost comparable levels of conidiation and the ability to prevent excessive cytoplasmic leakage upon hypotonic shock as the control strain (Fig. 2A,B). The transformants also had no apparent growth differences as compared with the control on either nutrient-rich or minimal media (data not shown). These data confirmed that the AoSO-EGFP fusion protein is functional and complements the loss of the native *Aoso* gene.

Accumulation of AoSO at the septal pore in response to stress

We sought to determine the subcellular localization of the AoSO-EGFP fusion protein expressed in the Δ*Aoso* strain in response to hyphal stress. Fluorescence microscopy revealed that under normal growth conditions, the AoSO-EGFP fusion protein was distributed throughout the cytoplasm and no obvious accumulation of AoSO at the septal pore was observed (Fig. 3A). In the subsequent experiments we focused on the first septum adjacent to the apical compartment as it was considered to be the most likely to allow cytoplasmic continuity between adjacent compartments, as suggested by van Peer et al. [19]. Since the Δ*Aoso* strain had reduced ability to prevent excessive cytoplasmic leakage upon hyphal injury, the subcellular localization of the AoSO-EGFP fusion protein was observed after subjected to hypotonic shock. The AoSO-EGFP fusion protein appeared as a punctate dot at the septal

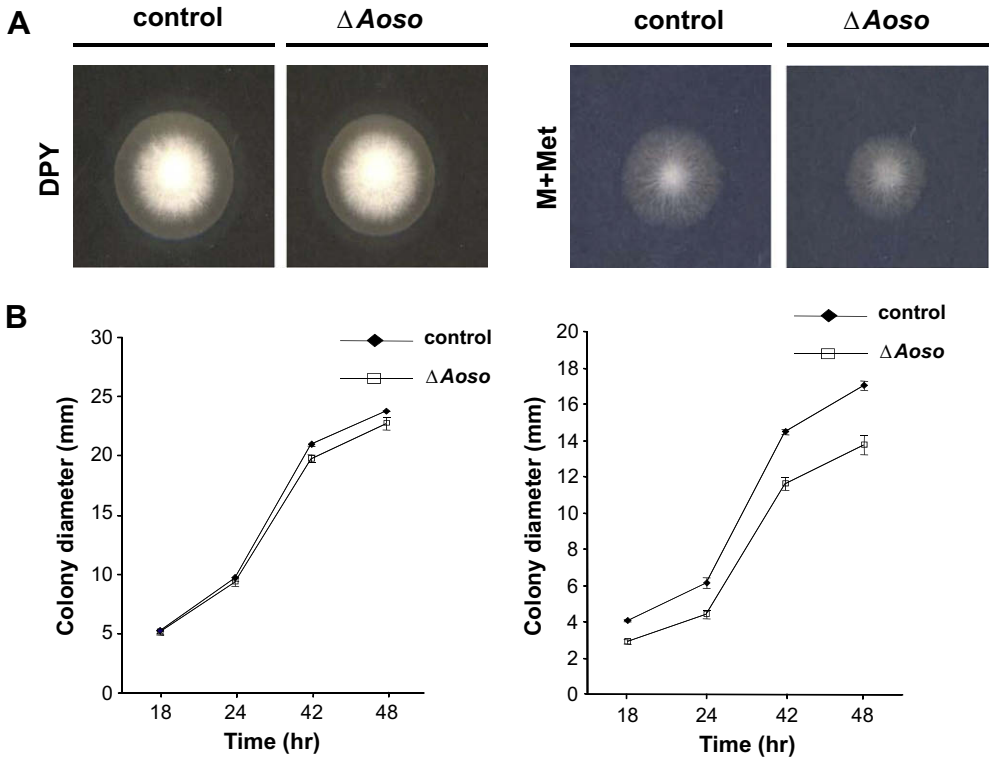

Fig. 1. Growth of the Δ*Aoso* strain on nutrient-rich (DPY) and minimal (M + Met) media. (A) Photographs of the mutant and control colonies after 2 days of growth at 30 °C. (B) Time-course growth analyses with respect to colony diameter.

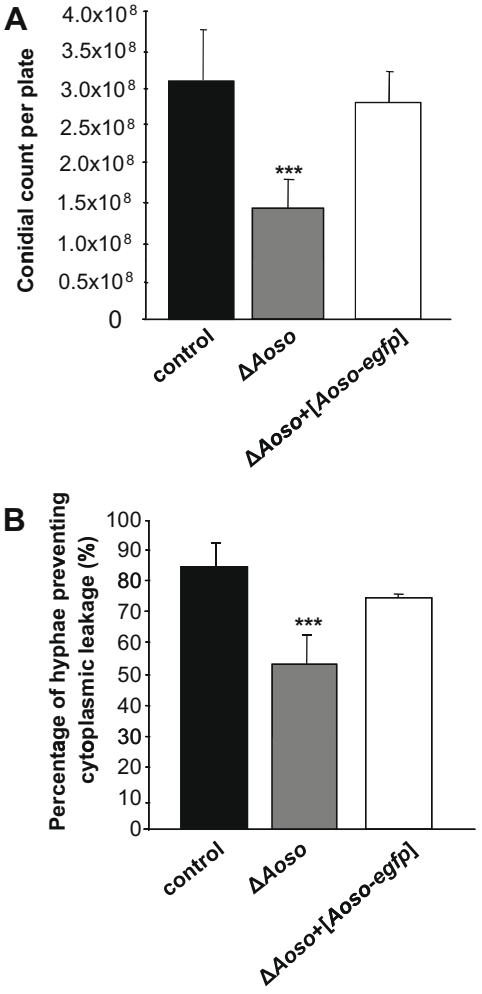

Fig. 2. Phenotypes of the Δ*Aoso* strain and the AoSO-EGFP fusion protein-complemented mutant. (A) Determination of the conidial number of each strain. The control, Δ*Aoso* strain, and Δ*Aoso* strain expressing the AoSO-EGFP fusion protein were grown on PD medium at 30 °C for 5 days. The number of conidia formed was counted and is shown in the graph. Standard deviations are indicated by error bars. *** represents the level of significance [$p < 0.001$ (t test, $n = 4$)]. (B) Excessive cytoplasmic leakage in the Δ*Aoso* strain upon hyphal tip bursting induced by hypotonic shock. The control, Δ*Aoso* strain, and Δ*Aoso* strain expressing the AoSO-EGFP fusion protein were subjected to hypotonic shock. The percentage of hyphae preventing excessive cytoplasmic leakage from the second compartment adjacent to the burst apical compartment upon hypotonic shock is shown. Fifty randomly selected hyphae with ruptured hyphal tips were observed. Error bars represent standard deviations. *** represents the level of significance [$p < 0.001$ (t test, $n = 4$)].

pore in the septum adjacent to the burst apical compartment (Fig. 3B), indicating that AoSO played a role in plugging the septal pore, which is a behavior similar to that observed for the Woronin body [9].

We further examined the subcellular localization of AoSO in response to various stress conditions. Hyphae grown at 30 °C for 18 h in liquid medium were then stressed by low (4 °C) and high (45 °C) temperatures, low (2) and high (10) pH, and nitrogen and carbon depletion. In response to each of these stresses, AoSO-EGFP fluorescence was observed as a punctate dot at the pore in the first septum (Fig. 3C). We estimated that the AoSO-EGFP fusion protein accumulated at the septal pore within 10–20 min of incubation at 4 °C and within 5 min at 45 °C. Time-lapse microscopy showed that the first appearance of AoSO-EGFP fluorescence at the septal pore appeared within approximately 20–25 min after the shift to acidic conditions (Fig. 3D). The other stress conditions, including alkaline pH and nitrogen and carbon depletion resulted in accumulation of the AoSO-EGFP fusion protein at the septal pore at almost the same rate as the low pH stress. Collectively, these data demonstrate that AoSO accumulates at the septal pore in response to a variety of stress conditions.

Finally, we examined the movement of AoSO-EGFP in response to physical stress, using pulse laser treatment to induce stress at a specific area along a hypha. The accumulation of the AoSO-EGFP fusion protein at the septal pore was then traced using time-lapse analysis. The advantage of this experimental system is that stress can be spatially targeted which is not possible using other environmental stresses. Pulse laser treatment was applied at a distance of 10–15 μm from the first septum in the strain expressing the AoSO-EGFP fusion protein without causing hyphal injury. Fluorescence microscopy showed that AoSO-EGFP fluorescence accumulated at the septal pore approximately 2 min after pulse laser treatment (Fig. 4) which indicates that this technique is an effective physical stress for inducing relocalization of AoSO to the septal pore.

Discussion

Filamentous ascomycetes are multicellular organisms interconnected via a septal pore that allows the passage of cytoplasm and organelles. Our experiments demonstrated that *A. oryzae* AoSO protein accumulated at the septal pore in response to several stresses. To our knowledge, this is the first report demonstrating the relocalization of a protein to the septal pore in response to stress in filamentous fungi.

Our analysis indicated that AoSO plays a role in the prevention of excessive cytoplasmic leakage by accumulating at the septal pore upon hyphal injury (Figs. 2B and 3B), which is similar to *N. crassa* SO protein [11]. However, the ratio of hyphae preventing excessive cytoplasmic leakage in the Δ*Aoso* strain was higher than that of the Woronin body-deficient strain, Δ*Aohex1* [9], and a double disruptant (Δ*Aoso* Δ*Aohex1*) did not show more severe cytoplasmic leakage than the Δ*Aohex1* strain (data not shown). Taken together, these results suggest that AoSO acts together with the Woronin body to plug the septal pore.

We found an interesting localization behavior of AoSO: it accumulated at the septal pore in response to various stresses (Figs. 3C and 4). Analysis with pulse laser treatment showed that early septal pore accumulation of AoSO occurred at a distance from the physically stressed hyphal region. In contrast, there are no reports of Woronin bodies plugging the septal pore under conditions of stress. AoSO also accumulated at the septal pore in the Δ*Aohex1* strain (data not shown), suggesting that its localization does not require the Woronin body. In order to determine how AoSO localizes site-specifically at the septal pore in response to stress, further experiments including screening for AoSO-interacting proteins are required. It was reported that *N. crassa* SO protein accumulated at the septal pore upon hyphal injury and remained there during the resumption of hyphal growth [11]. Likewise, AoSO was still observed at the septal pore after *A. oryzae* was relieved from the stress conditions of high temperature, low and high pH, nitrogen and carbon depletion, and pulse laser treatment. However, AoSO dissociated from the septal pore 20–25 min after the hyphae were relieved from the low temperature stress (data not shown). These localization data suggest the possibility that AoSO might regulate the septal pore connection between adjacent compartments in response to stress.

Recently, van Peer et al. [19] developed an experimental system in the basidiomycete *Schizophyllum commune* to evaluate cytoplasmic continuity between adjacent compartments by observation of the cytoplasmic streaming through the septal pore after injuring the hyphae. They suggested that the cytoplasmic continuity be-

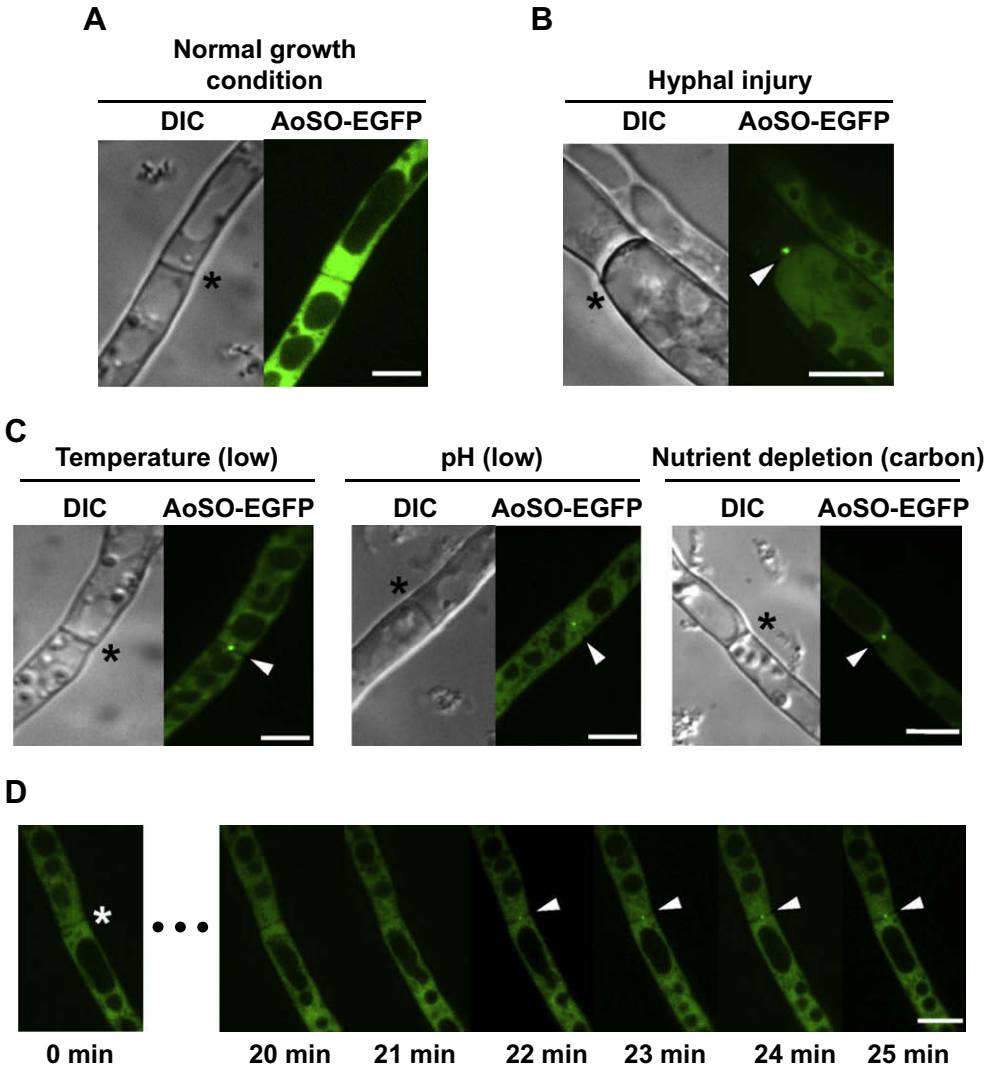

Fig. 3. Localization of the AoSO-EGFP fusion protein in *A. oryzae* hyphae. (A) DIC and fluorescence images of the AoSO-EGFP fusion protein under normal growth conditions. The Δ*Aoso* strain expressing the AoSO-EGFP fusion protein was grown in CD + Met liquid medium at 30 °C for 18 h. An asterisk denotes the first septum adjacent to the apical compartment. (B) Accumulation of the AoSO-EGFP fusion protein at the septal pore upon hyphal injury induced by hypotonic shock. Hyphae grown on agar medium were flooded with water to induce hyphal tip bursting. The hyphal region around the first septum adjacent to the burst apical compartment was observed by fluorescence microscopy. An asterisk denotes the septum while an arrowhead indicates the punctate dot formed at the septal pore. Note that the upper region of the hypha corresponds to the burst apical compartment. (C) Accumulation of the AoSO-EGFP fusion protein at the septal pore in response to various stresses. The Δ*Aoso* strain expressing the AoSO-EGFP fusion protein was grown in CD + Met liquid medium at 30 °C for 18 h and then subjected to stresses of temperature, pH, and nutrient depletion. The fluorescent micrographs of low (4 °C) temperature, low (2) pH, and carbon source depletion show the representative response to each kind of stress. Asterisks denote the first septa while arrowheads indicate punctate dots at the septal pore. (D) Time-lapse fluorescence microscopy of the AoSO-EGFP fusion protein accumulating at the septal pore upon subjecting Δ*Aoso* to low pH stress. Asterisks indicate the septum while arrowheads denote the AoSO-EGFP fusion protein accumulating at the septal pore as a punctate dot. Bars, 5 μm.

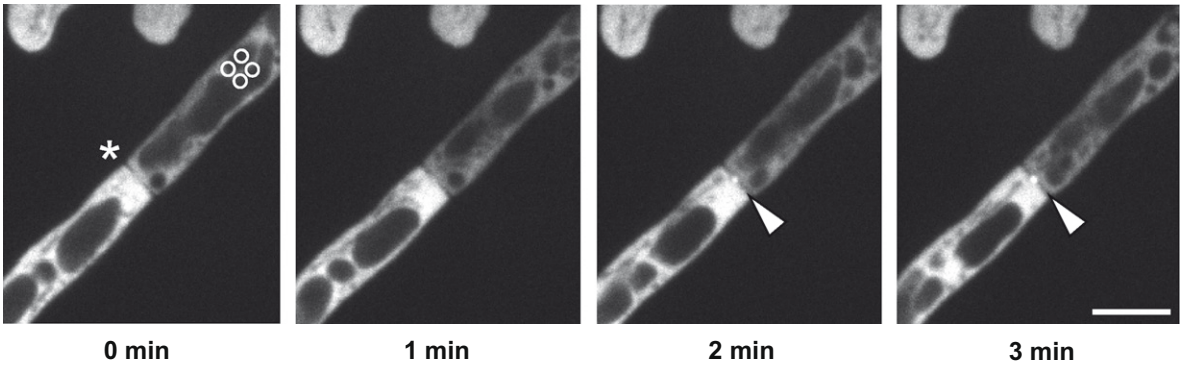

Fig. 4. Accumulation of the AoSO-EGFP fusion protein at the septal pore upon pulse laser treatment. The Δ*Aoso* strain expressing the AoSO-EGFP fusion protein was grown in CD + Met liquid medium at 30 °C for 18 h and then subjected to pulse laser treatment (0 min). The circles indicate the area where the pulse laser was applied, while arrowheads indicate the appearance of a punctate dot at the septal pore upon pulse laser treatment. Bar: 5 μm.

tween adjacent compartments was blocked by certain stress conditions, including high temperature [19]. This gating phenomenon of the septal pore in response to stress coincides well with the current stress-dependent behavior observed for AoSO localization and suggests that accumulation of AoSO at the septal pore serves to block cytoplasmic continuity between adjacent compartments. In contrast to *N. crassa* and *Sordaria fimicola*, which exhibit extensive cytoplasmic streaming through septal pores [4–6], such cytoplasmic streaming across the septum is rarely seen in *A. oryzae* (Maruyama, unpublished results). In order to monitor cytoplasmic continuity in living *A. oryzae* cells, it is first necessary to establish an appropriate experimental system. Further studies combining the identification and molecular characterization of other septal pore-localizing proteins and detailed observation of cytoplasmic continuity will help unravel the molecular mechanisms involved in the gating of septal pores in filamentous fungi.

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References

[1] V.A. Shepherd, D.A. Orlovich, A.E. Ashford, Cell-to-cell transport via motile tubules in growing hyphae of a fungus, J. Cell Sci. 105 (1993) 1173–1178.
[2] P. Markham, Occlusions of septal pores in filamentous fungi, Mycol. Res. 98 (1994) 1089–1106.
[3] M. Freitag, P.C. Hickey, N.B. Raju, E.U. Selker, N.D. Read, GFP as a tool to analyze the organization, dynamics and function of nuclei and microtubules in *Neurospora crassa*, Fungal Genet. Biol. 41 (2004) 897–910.
[4] W.K. Tey, A.J. North, J.L. Reyes, Y.F. Lu, G. Jedd, Polarized gene expression determines Woronin body formation at the leading edge of the fungal colony, Mol. Biol. Cell 16 (2005) 2651–2659.
[5] R.R. Lew, Mass flow and pressure-driven hyphal extension in *Neurospra crassa*, Microbiology 151 (2005) 2685–2692.
[6] S.K. Ng, F. Liu, J. Lai, W. Low, G. Jedd, A tether for Woronin body inheritance is associated with evolutionary variation in organelle positioning, PLoS Genet. 5 (2009) e1000521.
[7] P. Markham, A.J. Collinge, Woronin bodies of filamentous fungi, FEMS Microbiol. Rev. 46 (1987) 1–11.
[8] G. Jedd, N.H. Chua, A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane, Nat. Cell Biol. 2 (2000) 226–231.
[9] J. Maruyama, P.R. Juvvadi, K. Ishi, K. Kitamoto, 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 (2005) 1081–1088.
[10] A.P. Trinci, A.J. Collinge, Structure and plugging of septa of wild type and spreading colonial mutants of *Neurospora crassa*, Arch. Microbiol. 91 (1973) 355–364.
[11] A. Fleissner, N.L. Glass, SO, a protein envolved in hyphal fusion in *Neurospora crassa*, localizes to septal plugs, Eukaryot. Cell 6 (2007) 84–94.
[12] I. Engh, C. Würtz, K. Witzel-Schlömp, H.Y. Zhang, B. Hoff, M. Nowrousian, H. Rottensteiner, U. Kück, The WW domain protein PRO40 is required for fungal fertility and associates with Woronin bodies, Eukaryot. Cell 6 (2007) 831–843.
[13] A. Fleissner, S. Sarkar, D. Jacobson, M.G. Roca, N. Read, N.L. Glass, The SO locus is required for vegetative cell fusion and postfertilization events in *Neurospora crassa*, Eukaryot. Cell 4 (2005) 920–930.
[14] C.S. Escaño, P.R. Juvvadi, F.J. Jin, T. Takahashi, Y. Koyama, S. Yamashita, J. Maruyama, K. Kitamoto, Disruption of the Aopex11-1 gene envolved in peroxisome proliferation leads to impaired Woronin body formation is *Aspergillus oryzae*, Eukaryot. Cell 8 (2009) 296–305.
[15] K. Kitamoto, Molecular biology of the Koji molds, Adv. Appl. Microbiol. 51 (2002) 129–153.
[16] Y. Mabashi, T. Kikuma, J. Maruyama, M. Arioka, K. Kitamoto, Development of a versatile expression plasmid construction system for *Aspergillus oryzae* and its application to visualization of mitochondria, Biosci. Biotechnol. Biochem. 70 (2006) 1882–1889.
[17] F.J. Jin, T. Watanabe, P.R. Juvvadi, J. Maruyama, M. Arioka, K. Kitamoto, Double disruption of the proteinase genes, *tppA* and *pepE*, increases the production level of human lysozyme by *Aspergillus oryzae*, Appl. Microbiol. Biotechnol. 76 (2007) 1059–1068.
[18] S. Tada, K. Gomi, K. Kitamoto, K. Takahasbi, G. Tamura, S. Hara, Construction of a fusion gene comprising the taka-amylase A promoter and the *Escherichia coli* β-glucuronidase gene and analysis of its expression in *Aspergillus oryzae*, Mol. Gen. Genet. 229 (1991) 301–306.
[19] A.F. van Peer, W.H. Müller, T. Boekhout, L.G. Lugones, H.A. Wösten, Cytoplasmic continuity revisited: closure of septa of the filamentous fungus *Schizophyllum commune* in response to environmental conditions, PLoS One 4 (2009) e5977.