論文の内容の要旨

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論文題目

A study on the molecular and cellular mechanisms involved in septal pore plugging in *Aspergillus oryzae*

(麹菌 *Aspergillus oryzae* の隔壁孔制御に関する分子生物学的解析)

Filamentous ascomycetes are multicellular organisms that grow through polarized tip extension, resulting in tubular cells (hyphae) consisting of many compartments divided by septa. Neighboring compartments are interconnected via the septal pore, which allows the passage of cytoplasm and organelles between compartments. The intercellular communication via the septal pore may function to keep homeostasis as multicellular organisms. This characteristic is shared by higher eukaryotic organisms in gap junctions of animal cells and plasmodesmata of plant cells. However, the molecular mechanism regulating intercellular communication has not been intensively studied in filamentous ascomycetes.

In contrast, intercellular communication causes alarming risks when hyphae are lysed. To prevent this scenario, septal pores are plugged in response to hyphal injury, which is executed by a specialized organelle called the Woronin body. Although it was previously suggested that the Woronin body was an organelle derived from the peroxisome, the

relationship between peroxisomal proliferation/division and Woronin body differentiation had not been extensively investigated.

The main objective of this study is to evaluate the molecular and cellular processes associated with the septal pore in the filamentous fungus *Aspergillus oryzae*. The specific objectives are to investigate the role of peroxisomal proliferation/division in Woronin body formation; to characterize a septal-pore localizing protein, AoSO; and to determine the effect of calcium on the localization process of the protein.

1. Functional characterization of the role of the AoPex11 proteins in a septal pore plugging organelle, Woronin body[1)]

I examined whether Pex11 required for peroxisomal proliferation participates in Woronin body formation in *A. oryzae*. *A. oryzae* contained two orthologous *PEX11* genes that were designated as *Aopex11-1* and *Aopex11-2*. Deletion of the *Aopex11* genes revealed that only the Δ*Aopex11-1* strain showed reduced growth and enlarged peroxisomes in the presence of oleic acid as sole carbon source, indicating a defect in peroxisomal function and proliferation. Disruption of the *Aopex11-1* gene impaired the Woronin body function, leading to excessive loss of the cytoplasm upon hyphal injury. Dual localization analysis of peroxisome and Woronin body protein AoHex1 demonstrated that Woronin bodies fail to fully differentiate from peroxisomes in the Δ*Aopex11-1* strain. Furthermore, distribution of AoHex1 was found to be peripheral in the enlarged peroxisome or junctional in dumbbell-shaped peroxisomes. Electron microscopy of the Δ*Aopex11-1* strain revealed the presence of Woronin bodies that remained associated with organelles resembling peroxisomes, which was supported from

the sucrose gradient centrifugation confirming that the Woronin body protein AoHex1 overlapped with the density-shifted peroxisome in the Δ*Aopex11-1* strain. In conclusion, the present study implicated the role of Pex11 for Woronin body differentiation for the first time.

2. Functional and localization analyses of a septal pore plugging protein, AoSO

I hypothesized that filamentous ascomycetes need to have an acute system regulating intercellular communication in response to sudden environmental changes to keep homeostasis in multicellular organisms. I focused on an *A. oryzae* protein (AoSO) homologous to the *N. crassa* SO protein, which accumulates at the septal pore in aging. Cellular localization studies using EGFP showed that while the AoSO protein was not found at the septal pore during the growth in normal condition, but it was accumulated at the septal pore as a punctate dot under various stress conditions (high/low temperature, extreme acidic/alkaline pH, nitrogen/carbon starvation). This is the first observation showing that the AoSO protein is accumulated at the septal pore in response to specific stresses. Furthermore, it dissociated from the septal pore upon recovery from the stress, leading to a hypothesis that the AoSO protein may participate in regulation of intercellular communication in response to environmental stresses.

The Δ*Aoso* strain showed excessive loss of the cytoplasm during hyphal injury like the Woronin body-deficient strain (Δ*Aohex1*). This is consistent with the result that the AoSO protein was also accumulated at the septal pore during hyphal injury. Thus, the AoSO protein contributes to preventing cytoplasmic bleeding upon hyphal injury.

3. Effect of calcium on AoSO accumulation at the septal pore

Calcium plays an important role as an intracellular signaling molecule in animals, plants and fungi. In order to determine the effect of calcium on AoSO localization upon stress, pulse laser treatment was employed, in which the AoSO protein was accumulated as a punctate dot at the septal pore when a part of hyphae near the septum was treated with pulse laser. When the concentration of extracellular calcium was increased, the accumulation of the AoSO protein at the septal pore upon the stress was hastened. Addition of calcium chelators (BAPTA-AM and EGTA) and cyclosporin A (calcineurin inhibitor) caused a delay in the accumulation of the AoSO protein at the septal pore. Absence of the stretch-activated calcium channel, AoMid1, also resulted in a delay in accumulation of the AoSO protein at the septal pore upon pulse laser stimulation. Furthermore, accumulation of the AoSO protein at the septal pore was apparent in the Woronin body-deficient strain (Δ*Aohex1*) although it was delayed, which was suppressed by increasing the concentration of extracellular calcium. These data suggested the involvement of calcium signaling pathway for the AoSO accumulation process at the septal pore.

Reference

1) **C. S. Escaño, P. R. Juvvadi, F. J. Jin, T. Takahashi, Y. Koyama, S. Yamashita, J. Maruyama, and K. Kitamoto** (2009). Disruption of the *Aopex11-1* gene involved in peroxisome proliferation leads to impaired Woronin body formation in *Aspergillus oryzae*. *Eukaryotic Cell.* 8:296-305.