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Mini-Review:

Investigation of the biological roles of autophagy in appressorium morphogenesis in *Magnaporthe oryzae**

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Abstract: *Magnaporthe oryzae* has been used as a primary model organism for investigating fungus-plant interaction. Many researches focused on molecular mechanisms of appressorium formation to restrain this fungal pathogen. Autophagy is a very high conserved process in eukaryotic cells. Recently, autophagy has been considered as a key process in development and differentiation in *M. oryzae*. In this report, we present and discuss the current state of our knowledge on gene expression in appressorium formation and the progress in autophagy of rice blast fungi.

Key words: *Magnaporthe oryzae*, Appressorium, Gene expression, Autophagy
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INTRODUCTION

A model fungus, *Magnaporthe oryzae* causing rice blast disease, employs an appressorium with a well evolved dome-shaped cellular structure, in the course of interaction with the rice host, in order to effectively penetrate different rice tissues, i.e., leaves, stems and roots (Ou, 1985). To understand the molecular mechanisms of appressorium formation to restrain this fungal pathogen, considerable progress and efforts have been made to identify gene functions, which is necessary for the regulation of appressorium-mediated penetration events in the development and pathogenicity of *M. oryzae*. So far, several signal pathways related with this process have been extrapolated in *M. oryzae*, including the cAMP (cyclicadenylicacid) respond process, the mitogen-activated protein kinase cascade, the GTP (guanosine triphosphate)-binding (G) protein transduction pathway and the calcium-mediated signal transduction (Dean *et al*., 2005; Lee and Dean, 1993; Liu and Dean, 1997; Nguyen *et al*., 2008; Xu and Hamer, 1996). Currently, autophagy was confirmed to be an important pathway to the turgor accumulation in appressorium (Liu *et al*., 2007; Veneault-Fourrey *et al*., 2006). However, molecular mechanisms in these events in this fungus have not been fully characterized. Here we report the biological roles of autophagy pathways in appressorium formation.

GENES SPECIFICALLY EXPRESSED IN APPRESSORIUM FORMATION

We have developed a novel method for purification of total RNA from appressoria of *M. oryzae*. The total RNA of appressoria was isolated directly from the appressoria of this fungus inoculated on a hydrophobic surface of projection transparency film (terylene resin membrane, Gaoke, China) with Trizol reagent (Lu *et al*., 2005a; 2005b). We had successfully obtained the total RNA of *M. oryzae* from the appressoria incubated (from conidia) for 2, 4, 8, 12,

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18, 24 and 30 h and built mature appressorium cDNA libraries (Liu *et al*., 2008). Total RNA of the conidia/aerial mycelia/substrate mycelia mixture and the mature appressoria at the incubation of 23.5~24.5 h were isolated. A subtractive appressoria cDNA library, subtracted by the conidia/aerial mycelia/substrate mycelia mixture, was constructed by suppression subtractive hybridization (SSH). Among 142 ESTs (expressed sequence tags) selected, 70 were described previously and another 72 were first reported in *M. oryzae*. Eighty-nine percent of the ESTs (126/142) had significant matches (P<0.001) to known (or predicted) genes present in GenBank at the time of submission. All 142 identified genes were functionally classified: 12 (8.45%) ESTs in fat metabolism such as cytochrome P450, keto acyl reductase, acyl-CoA dehydrogenase, autophagy pathway, HMGB (high mobility group B) family and C-14 sterol reductase; 10 (7.04%) in protein modification such as serine-theronine kinase, oligosaccharyltransferase alpha subunit, and cysteine proteinase; 10 (7.04%) in nucleic acid metabolism; 8 (5.63%) in sugar metabolism; 6 (4.23%) in antibiotic metabolism; 4 (2.83%) in protein synthesis; 1 (0.70%) in cell wall; and 21 (14.79%) in other functions, whereas 70 (49.30%) ESTs were classified as having unknown function. We also used the reverse-transcript polymerase chain reaction (RT-PCR) analysis to confirm differential gene expression in appressoria and conidia/aerial mycelia/substrate mycelia mixture as indicted by SSH subtraction strategy. These results reveal differential gene expression in the appressorium stage of *M. oryzae*. Functional analyses of 35 novel genes related to this stage are being carried on now, and some of them have been reported, such as *Mnh6* (Lu *et al*., 2007), *MgATG1* (Liu *et al*., 2007), and *Mtp1* (Lu *et al*., 2008).

AUTOPHAGY PATHWAY LINKED TO APPRESSORIUM MATURATION AND PENETRATION

Autophagy is a ubiquitous and evolutionarily conserved process that degrades and recycles long-lived proteins and organelles, occurring in all eukaryotic cells (Levine and Klionsky, 2004). It is an intracellular, bulk degradation process in which cytosol and organelles are sequestered within double-membrane vesicles termed autophagosomes that deliver the contents to the lysosome/vacuole for degradation and recycle of the macromolecules (Yorimitsu and Klionsky, 2005). For many years, autophagy has been presumed to be involved in cellular architectural changes that occur during differentiation and development, presumably via its role in organelle and protein turnover (Klionsky, 2005; Levine and Klionsky, 2004).

An EST (GenBank accession No. CK828251) encoding *MgATG1*, a serine/threonine kinase required for autophagy, was found in screening genes highly expressed during appressorium stage from the subtractive suppressive cDNA library of the 24-h mature appressoria. To find out the potential role of *MgATG1* during appressorium formation, maturation and penetration, we isolated a full-length of *MgATG1* gene from genomic DNA of *M. oryzae* Guy11 and appressorium stage cDNA library by long distance PCR. The 2949-bp cDNA fragment containing full coding DNA sequences (CDS) was cloned to pUCm-T (Sangon, Shanghai, China) and sequenced (GenBank accession No. DQ224381).

MgATG1 in *M. oryzae*, containing an S_TKc domain of 256 residues, is a 982-amino acid serine/threonine protein kinase of 108 kDa. The alignment between MgATG1 of rice blast fungus and other ATG1 of other organisms shows very high homologies. Significantly, ATG1 in *M. oryzae* is 60% identical to CLK1 (homolog of ATG1 in yeast) in *Colletotrichum lindemuthianum* (Dufresne *et al*., 1998), 56% to ATG1 (PDD7p) in *Hansenula polymorpha* (Komduur *et al*., 2003), 51% to ATG1 (APG1/AUT3) in *Saccharomyces cerevisiae* (Matsuura *et al*., 1997), and 36% to ULK1 in *Mus musculus* (Yan *et al*., 1998), ULK1 *Homo sapiens* (Kuroyanagi *et al*., 1998) and UNC-51 in *Caenorhabditis elegans* (Ogura *et al*., 1994). The Δ*mgatg1* mutant of *M. oryzae*, in which *MgATG1* gene was deleted by targeted gene replacement, shows a defect in autophagy and also exhibits defects in morphogenesis including reduction in conidiation, delay in conidia germination, and reduction in appressorium turgor. As a result, the *MgATG1* disruption mutant loses its pathogenicity to two tested host plants, rice CO-39 and barley ZJ-8. These phenotypes were recovered by re-introduction of an intact copy of *MgATG1* to Δ*mgatg1* null mutant.

Taken together, *MgATG1* gene is essential in autophagy and is also required for development and pathogenicity of *M. oryzae* (Liu *et al*., 2007).

AUTOPHAGY RELATED GENES IN *M. ORYZAE*

Currently, we have isolated 21 autophagy genes homologous to yeast, which are highly conserved among other eukaryotes, including humans and plants. Disruption of other autophagy related genes, such as *MgATG2* (GenBank accession No. EU984499), *MgATG4*, *MgATG5* (GenBank accession No. EF486491), *MgATG8*, *MgATG9* and *MgATG18* (GenBank accession No. EU984500) involved in the autophagy pathway, influenced the capability of surviving starvation, conidiation, conidial germination, lipid turnover, and appressorium turgor generation in *M. oryzae*. As a result, these null mutants lose their penetration and pathogenicity into the host plants. By reintroducing the autophagy genes to their null mutants, all the defects were restored. However, targeted deletion of *MgATG11*, *MgATG13* and *MgATG17* homologous to *ATG11*, *ATG13* and *ATG17* in yeast respectively, did not block autophagy pathway in *M. oryzae* (Liu *et al*., 2008), suggesting that there were different protein components involving in the autophagy machinery between two of these fungi. Taken together, clarification of the functions and network of these autophagy genes will lead to a better understanding of the role of autophagy genes in the pathogenesis of this fungus to control this fungal disease, and to the discovery of novel autophagy genes by using this model filamentous fungus.

CONCLUSION

In this review, we summarize gene expression in appressorium formation and emphasize the autophagy pathway in *M. oryzae*. Studies on autophagy related genes have revealed numerous effects involved in every stage of development and differentiation in *M. oryzae*. Future studies need to address interaction of these autophagy related genes and molecular mechanisms of autopahgy in the rice blast fungus. It is also necessary to address the function of autophagy in cell development and differentiation in the fungus. The studies on autophagy machinery in *M. oryzae*, an attractive model system, will lead to unraveling the functions of autophagy in other filamentous fungi.

References

Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thon, M., Kulkarni, R., Xu, J.R., Pan, H., *et al*., 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature*, **434**(7036):980-986. [doi:10.1038/nature03449]

Dufresne, M., Bailey, J.A., Dron, M., Langin, T., 1998. clk1, a serine/threonine protein kinase-encoding gene, is involved in pathogenicity of *Colletotrichum lindemuthianum* on common bean. *Mol. Plant Microbe Interact.*, **11**(2):99-108. [doi:10.1094/MPMI.1998.11.2.99]

Klionsky, D.J., 2005. The molecular machinery of autophagy: unanswered questions. *J. Cell Sci.*, **118**(Pt 1):7-18. [doi:10.1242/jcs.01620]

Komduur, J.A., Veenhuis, M., Kiel, J.A., 2003. The *Hansenula polymorpha* PDD7 gene is essential for macropexophagy and microautophagy. *FEMS Yeast Res.*, **3**(1):27-34. [doi:10.1016/S1567-1356(02)00135-6]

Kuroyanagi, H., Yan, J., Seki, N., Yamanouchi, Y., Suzuki, Y., Takano, T., Muramatsu, M., Shirasawa, T., 1998. Human ULK1, a novel serine/threonine kinase related to UNC-51 kinase of *Caenorhabditis elegans*: cDNA cloning, expression, and chromosomal assignment. *Genomics*, **51**(1):76-85. [doi:10.1006/geno.1998.5340]

Lee, Y.H., Dean, R.A., 1993. cAMP regulates infection structure formation in the plant pathogenic fungus *Magnaporthe grisea*. *Plant Cell*, **5**(6):693-700. [doi:10.1105/tpc.5.6.693]

Levine, B., Klionsky, D.J., 2004. Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev. Cell*, **6**(4):463-477. [doi:10.1016/S1534-5807(04)00099-1]

Liu, S., Dean, R.A., 1997. G protein alpha subunit genes control growth, development, and pathogenicity of *Magnaporthe grisea*. *Mol. Plant Microbe Interact.*, **10**(9):1075-1086. [doi:10.1094/MPMI.1997.10.9.1075]

Liu, T.B., Lu, J.P., Liu, X.H., Min, H., Lin, F.C., 2008. A simple and effective method for total RNA isolation of appressoria in *Magnaporthe oryzae*. *J. Zhejiang Univ. Sci. B*, **9**(10):811-817. [doi:10.1631/jzus.B0860011]

Liu, X.H., Lu, J.P., Zhang, L., Dong, B., Min, H., Lin, F.C., 2007. Involvement of a *Magnaporthe grisea* serine/threonine kinase gene, *MgATG1*, in appressorium turgor and pathogenesis. *Eukaryot. Cell*, **6**(6):997-1005. [doi:10.1128/EC.00011-07]

Lu, J.P., Liu, T.B., Lin, F.C., 2005a. Identification of mature appressorium-enriched transcripts in *Magnaporthe grisea*, the rice blast fungus, using suppression subtractive hybridization. *FEMS Microbiol. Lett.*, **245**(1):131-137. [doi:10.1016/j.femsle.2005.02.032]

Lu, J.P., Liu, T.B., Yu, X.Y., Lin, F.C., 2005b. Representative appressorium stage cDNA library of *Magnaporthe grisea*.

J. Zhejiang Univ. Sci. B, **6**(2):132-136. [doi:10.1631/jzus.2005.B0132]

Lu, J.P., Feng, X.X., Liu, X.H., Lu, Q., Wang, H.K., Lin, F.C., 2007. Mnh6, a nonhistone protein, is required for fungal development and pathogenicity of *Magnaporthe grisea*. *Fungal Genet. Biol.*, **44**(9):819-829. [doi:10.1016/j.fgb.2007.06.003]

Lu, Q., Lu, J.P., Li, X.D., Liu, X.H., Min, H., Lin, F.C., 2008. *Magnaporthe oryzae* MTP1 gene encodes a type III transmembrane protein involved in conidiation and conidial germination. *J. Zhejiang Univ. Sci. B*, **9**(7):511-519. [doi:10.1631/jzus.B0820015]

Liu, X.H., Lu, J.P., Dong, B., Lin, F.C., 2008. Autohagy and the cvt pathway in *Magnaporthe oryzae*. Unpublished manuscript.

Matsuura, A., Tsukada, M., Wada, Y., Ohsumi, Y., 1997. Apg1p, a novel protein kinase required for the autophagic process in *Saccharomyces cerevisiae*. *Gene*, **192**(2):245-250. [doi:10.1016/S0378-1119(97)00084-X]

Nguyen, Q.B., Kadotani, N., Kasahara, S., Tosa, Y., Mayama, S., Nakayashiki, H., 2008. Systematic functional analysis of calcium-signalling proteins in the genome of the rice-blast fungus, *Magnaporthe oryzae*, using a high-throughput RNA-silencing system. *Mol. Microbiol.*, **68**(6):1348-1365. [doi:10.1111/j.1365-2958.2008.06242.x]

Ogura, K., Wicky, C., Magnenat, L., Tobler, H., Mori, I., Muller, F., Ohshima, Y., 1994. *Caenorhabditis elegans* unc-51 gene required for axonal elongation encodes a novel serine/threonine kinase. *Genes. Dev.*, **8**(20):2389-2400. [doi:10.1101/gad.8.20.2389]

Ou, S.H., 1985. Rice Diseases, 2nd Ed. Commonwealth Mycological Institute, Kew, UK.

Veneault-Fourrey, C., Barooah, M., Egan, M., Wakley, G., Talbot, N.J., 2006. Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science*, **312**(5773):580-583. [doi:10.1126/science.1124550]

Xu, J.R., Hamer, J.E., 1996. MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea*. *Genes. Dev.*, **10**(21):2696-2706. [doi:10.1101/gad.10.21.2696]

Yan, J., Kuroyanagi, H., Kuroiwa, A., Matsuda, Y., Tokumitsu, H., Tomoda, T., Shirasawa, T., Muramatsu, M., 1998. Identification of mouse ULK1, a novel protein kinase structurally related to *C. elegans* UNC-51. *Biochem. Biophys. Res. Commun.*, **246**(1):222-227. [doi:10.1006/bbrc.1998.8546]

Yorimitsu, T., Klionsky, D.J., 2005. Autophagy: molecular machinery for self-eating. *Cell Death Differ.*, **12**(Suppl. 2):1542-1552. [doi:10.1038/sj.cdd.4401765]