Novel conidiation regulatory mechanism by N-end rule pathway in *Aspergillus oryzae*

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Research objective

The stability of intracellular proteins depends on their N-terminal amino acid residues. In eukaryotes, the ubiquitin ligase Ubr1 generally recognizes intracellular proteins, whose N-terminal amino acids are arginine, lysine, or histidine, leading to their degradation by the proteasome. This protein degradation mechanism, called the N-end rule, is involved in various cellular processes, such as the regulation of peptide transporter gene expression. We found that disruption of the Ubr1 ortholog gene (*ubrA*) in *Aspergillus oryzae* markedly enhances conidiospore formation. There have been no reports on the N-end rule in filamentous fungi, and its involvement in conidiospore formation is unknown. In this study, we analyzed the N-end rule in *A. oryzae* and its involvement in the degradation of transcription factors that control conidiospore formation.

Methods

To examine whether the N-end rule is applicable in *A. oryzae*, we expressed ubiquitin-fused green fluorescent proteins (GFPs)[(1)], which can express GFP with different N-terminal amino acids, in wild-type and *ubrA* disruption strains. GFP protein levels were compared in the cells by western blot analysis using an anti-GFP antibody. In addition, to investigate whether the N-end rule is involved in the degradation of transcription factors that control conidiospore formation, the transcription factors fused 3×FLAG tag to the C-terminus were expressed in wild-type and *ubrA* disruption strains. The amounts of these transcription factors in the cells were compared by western blot analysis using an anti-FLAG antibody.

Results

Analysis of N-end rule in *A. oryzae*

GFPs with N-terminal amino acids methionine or arginine were expressed as a fusion protein with ubiquitin in *A. oryzae*. Since the ubiquitin of this fusion protein is cleaved by deubiquitinating enzymes, this allowed us to examine the expression level of GFP with different N-terminal amino acids. In the wild-type strain, only GFP with N-terminal

methionine (M-GFP) was detected, whereas both M-GFP and GFP with N-terminal arginine (R-GFP) were detected in *the ubrA* disruption strain. These results indicate that R-GFP was degraded in a UbrA-dependent manner, indicating that the N-end rule is applicable to *A. oryzae*.

Involvement of the N-end rule in degradation of transcription factors that regulate the conidiospore formation

Conidiospore formation in filamentous fungi is mainly regulated by the master regulator BrlA. Two transcription start sites of the *brlA* gene provide two types of translation products: BrlAα and BrlAβ. The N-terminus of BrlAβ is extended 23 amino acids from that of Brlα. Each BrlA with a C-terminal 3×FLAG tag was expressed in the wild-type and *ubrA* disruption strains. Western blot analysis revealed no significant difference in the abundance of a protein of approximately 50 kDa, which is presumed to be the full-length translational product of *brlA*. In contrast, fragments of approximately 30 kDa of both BrlA proteins were also detected. These fragments accumulated more in the *ubrA* disruption strain than in the wild strain. These results suggest that both BrlAa and BrlAb are cleaved, and that their C-terminal cleavage fragments are degraded in a UbrA-dependent manner.

Conclusion

This study provides the first evidence that the N-end rule is applicable to filamentous fungi. We also showed that BrlA is cleaved near the middle, and that the resulting C-terminal fragment may be degraded in a UbrA-dependent manner. Since the DNA-binding domain of BrlA is located near the C-terminus, we speculate that the accumulated C-terminal fragment of BrlA promotes conidiospore formation in the *ubrA* disruption strain. Future studies will be performed to verify whether the C-terminal cleavage fragment of BrlA maintains transcriptional ability and to identify the cleavage site of BrlA. The findings will elucidate the regulatory mechanism of conidiospore formation via the N-end rule.

References

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