A decrease in secretory protein mRNAs under endoplasmic reticulum stress in filamentous fungi: Elucidation of the mechanism and application in protein secretory production

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Research objectives

When eukaryotic cells are exposed to dithiothreitol (DTT) or tunicamycin, unfolded proteins are accumulated in the endoplasmic reticulum (ER), thereby activating transcription of the ER chaperone genes. This phenomenon is called unfolded protein response (UPR). In addition to the UPR, filamentous fungi undergo a downregulation of gene expression of secreted proteins, called as repression under secretion stress (RESS), when treated with DTT[1]. Although RESS might be caused by the transcriptional repression of secretory protein genes depending on their promoter sequences, the detail remains unclear.

We have shown that the UPR was activated in *Aspergillus oryzae* under the conditions inducing the production of amylolytic enzymes and in *A. oryzae* by producing an aberrant secretory protein[2,3]. These ER stresses could cause negative feedback on protein production through RESS. Therefore, we aimed to elucidate the RESS mechanism in *A. oryzae*.

Methods

Detection of RESS

A. oryzae strains were grown in Czapek-Dox (CD) liquid medium containing 1% fructose and 0.1% peptone at 30ºC for 24 h, and then transferred to the same medium containing 1% maltose instead of fructose, to induce an α-amylase expression. DTT was added to the culture at a final concentration of 20 mM and the mycelia were harvested after appropriate periods. Total RNAs were prepared from the mycelia and subjected to northern blot analyses. In order to express *ireA* under the control of thiamine-repressible *nmtA* promoter, media containing 10 μM thiamine were used.

Results

1) Involvement of a promoter-dependent transcription repression in the decrease of α-amylase mRNA

The amount of *amyA/B/C* (α-amylase) mRNAs was dramatically decreased in the wild type strain in response to DTT treatment. Since a transcript of *amyR*, which encodes a

transcriptional activator for *amyA/B/C* expression, was gradually decreased in this condition, it was suggested that the RESS of *amyA/B/C* could be due to the transcriptional inactivation of *amyA/B/C* associated with the downregulation of AmyR. However, the RESS of *amyB* was observed even when *amyB* was expressed under the control of the promoter of *enoA* (enolase gene), whose expression is not influenced by AmyR. These results suggested that the promoter-dependent transcriptional repression was not the leading cause of the RESS of *amyA/B/C.*

2) The *amyA/B/C* mRNAs are degraded dependently on IreA under ER stress

Nascent polypeptides being synthesized in the cytoplasm are targeted to the ER membrane as a part of the tertiary complex with a ribosome and an mRNA. Therefore, it was assumed that the mRNA of a secretory protein being synthesized might be destabilized by the stressed ER. Accordingly, we constructed the strain that expressed the AmyB containing frame shift mutation in its ER targeting signal (AmyB-FS) and analyzed the amount of the *amyB-FS* mRNA under the ER stress condition. We found that the decrease in the amyB mRNA was evaded by this mutation, suggesting that the decrease in the *amyB* mRNA was largely due to the ER targeting of the mRNA.

Next, we investigated whether this phenomenon was regulated by the UPR. Under the ER stress condition, the mRNA of bZIP-type transcription factor HacA undergoes unconventional splicing by the ER membrane-spanning kinase/endoribonuclease IreA to induce the UPR[4]. Accordingly, we analyzed the RESS in the strain repressing *ireA* and found that the decrease in the *amyA/B/C* mRNAs was evaded by *ireA* repression. Similarly, the *amyA/B/C* mRNA decrease was very limited in the Δ*ireA* cells expressing the spliced form of HacA. These results suggested that the RESS may not be a downstream of the UPR, but directly or indirectly regulated by IreA. Therefore, we assumed that the *amyA/B/C* mRNAs were degraded in an IreA-dependent manner when the cells were treated with DTT. To test this hypothesis, we attempted to detect the degradation products of the *amyA/B/C* mRNAs in the ER stress condition. If an mRNA is cleaved by an endoribonuclease, the resulting mRNA fragments are degraded by exoribonucleases. To detect such mRNA fragments, we analyzed the RESS in the strain deleted for *ski2*, which encodes a subunit of the exoribonuclease complex, exosome. Northern blot analysis revealed that the *amyA/B/C* mRNA fragments appeared after the DTT treatment, suggesting that mRNA decay might be involved in the downregulation of the *amyA/B/C* mRNAs.

Conclusion

In this study, we showed that mRNA decay was involved in the downregulation of gene expression of secretory proteins under ER stress elicited with DTT. We assume that the mRNA targeted to the ER membrane as a complex with the nascent polypeptides and

ribosomes may undergo cleavage by IreA and the resulting mRNA fragments may be degraded by exosomes. The decay of the mRNAs of secretory proteins under ER stress has been reported in *Drosophila melanogaster* for the first time and named as "regulated Ire1-dependent decay (RIDD)"[5]. RIDD was also found in mammals and plants. Our study suggested that filamentous fungi also utilize the RIDD mechanism to downregulate gene expression of secretory proteins. If the ER stress elicited by the overexpression of exogenous secretory proteins activates RIDD, it could be a bottleneck in protein production with filamentous fungi. It is necessary to analyze whether the overexpression of exogenous secretory proteins induces RIDD in filamentous fungi.

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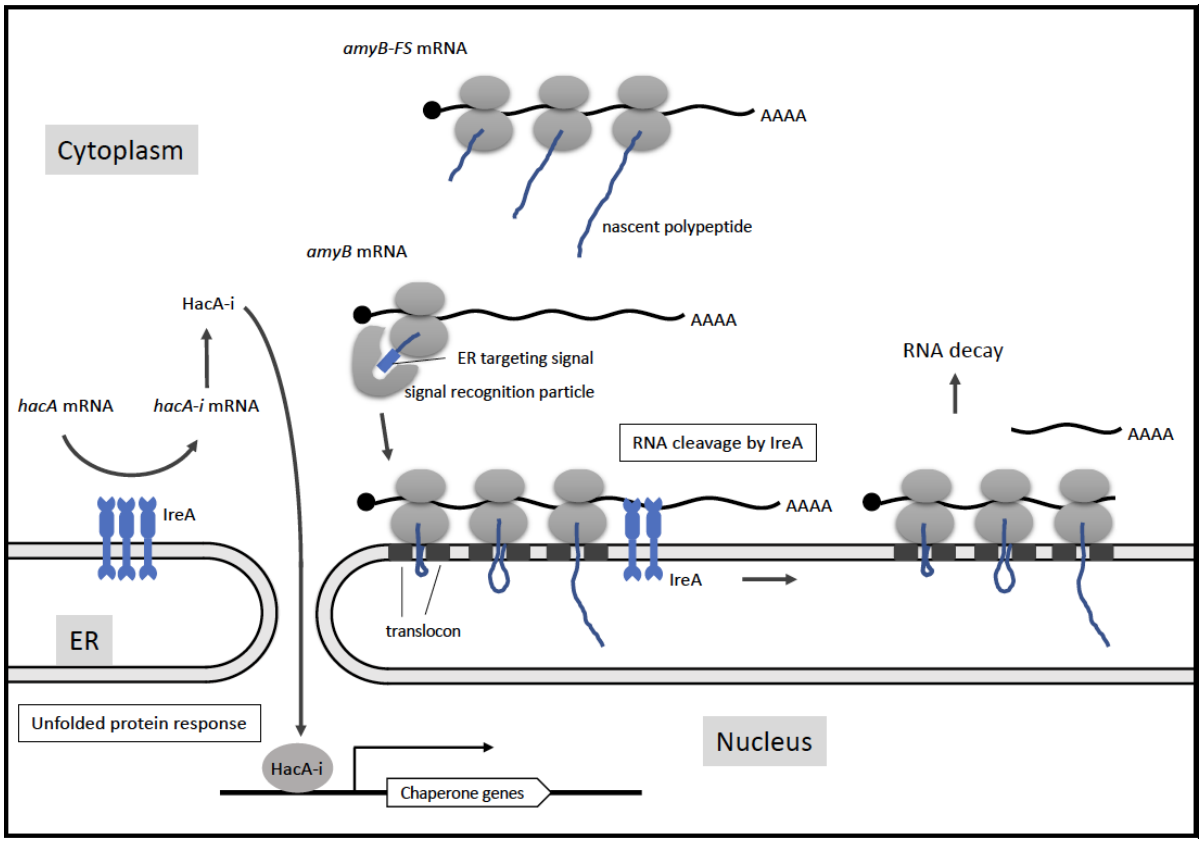

Figure A model of *amyB* mRNA decay induced by dithiothreitol treatment

Wild type *amyB* mRNA is recruited to the endoplasmic reticulum (ER) membrane dependently on the ER targeting signal of nascent polypeptide and undergoes degradation. The *amyB-FS* mRNA containing the frame shift mutation in its sequence coding the ER targeting signal is unable to be targeted to the ER and thus evades its degradation.

References

1) Pakula, T. M., Laxell, M., Huuskonen, A., Uusitalo, J., Saloheimo, M. and Penttilä, M. (2003) The effects of drugs inhibiting protein secretion in the filamentous fungus Trichoderma reesei. Evidence for down-regulation of genes that encode secreted proteins in the stressed cells, *J. Biol. Chem.*, **278:** 45011-45020.
2) Tanaka, M., Shintani, T. and Gomi, K. (2015) Unfolded protein response is required for Aspergillus oryzae growth under conditions inducing secretory hydrolytic enzyme production, *Fungal Genet. Biol.*, **85:** 1-6.
3) Yokota, J. I. *et al.* (2017) Cellular responses to the expression of unstable secretory proteins in the filamentous fungus *Aspergillus oryzae*, *Appl. Microbiol. Biotechnol.*, **101:** 2437-2446.
4) Saloheimo, M., Valkonen, M. and Penttilä, M. (2003) Activation mechanisms of the HACI-mediated unfolded protein response in filamentous fungi, *Mol. Microbiol.*, **47:** 1149-1161.
5) Hollien, J. and Weissman, J. S. (2006) Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response, *Science*, **313:** 104-107.