Mechanism for mRNA maturation control in basidiomycetes

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Research aims

Basidiomycete fungi have been the target of interesting research for their wide application including edible use as well as industrial utilization in the production of bioactive compounds, lectins, drug intermediates, fermentation foods, pretreatment of lignocelluloses for biomass refinery, and bioremediation of polluted environments. Recently, it has been reported that basidiomycetes lack most of the carbohydrate-modifying system present in other eukaryotic organisms, and we are attempting to develop a biopharmaceutical glycoprotein production system in the oyster mushroom, *Pleurotus ostreatus*, using recombinant gene expression combined with in vitro glycoengineering techniques.

Most studies on recombinant protein production using eukaryotic microorganisms have been done in ascomycete fungi such as *Saccharomyces cerevisiae* and *Aspergillus oryzae*. However, there is little compatibility between ascomycete and basidiomycete expression vectors. Moreover, even when homologous promoter sequences are used to drive codon-optimized coding sequences, the expression of heterologous proteins in basidiomycetes is still challenging. Elucidating the fundamental mechanisms for gene expression control in basidiomycete fungi is paramount for their utilization as an industrial microorganism. The aim of this research is to demonstrate the unique properties of the mRNA maturation process in basidiomycetes in order to develop a reliable system for heterologous protein expression in these fungi.

Methods

We have developed a protoplast transfection system in the basidiomycete that makes it possible to precisely evaluate the activity of each gene expression control system using a transient reporter gene. It has been difficult to compare the expression of reporter constructs in a stable transformation system because the introduced gene constructs are integrated with different copy numbers at random sites in the chromosome of each transformant. Using the protoplast transfection system, a basic promoter structure was determined for *gpd* and *ß-tub* genes in *Ceriporiopsis subvermispora* (to be published). In the following sections of this report, we describe the experimental results for the characterization of each step in the

basidiomycete mRNA maturation process.

Results

① Function of introns

Stable transformation experiments in *Schizophyllum commune* have demonstrated that an intron is essential for efficient gene expression in this organism [1)]. This has become an established theory in basidiomycetes. We tested the function of introns in recombinant gene expression by analyzing the expression of a transient gene transfected into *C. subvermispora*. The results showed that there is a negligible difference in the expression of reporter gene constructs with or without an intron, indicating that an intron is therefore dispensable for effective gene expression in this fungus.

② Evaluation of terminator sequences

Generally, mRNA processing starts during transcription but it has not been described whether transcription terminators have an important role in basidiomycete gene expression. Functional differences among terminator sequences have been mostly ignored in the design of expression vectors. We demonstrated there is differential gene expression of reporter gene constructs harboring different terminators, such as the *gpd* and *ß-tub* terminators, in *C. subvermispora* (Figure 1). These results suggest that to construct more powerful expression cassettes, the use of a strong terminator is recommended.

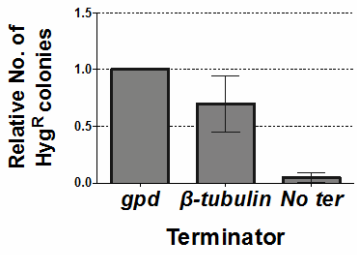

Figure 1. Transfection analysis of Hyg^R maker plasmid with different terminator sequences.
No ter: no terminator sequence.

③ Analysis of the poly(A) signal

In filamentous fungi, it was reported that poly(A) signals are less conserved than those in mammalian species and a series of AT-rich stretches can act as poly(A) signal in *Aspergillus oryzae*[2)]. If AT-rich sequences can similarly act as poly(A) in basidiomycetes, they could represent an obstacle for the production of full-length mRNAs coding for heterologous proteins. To our knowledge, poly(A) signals have never been investigated

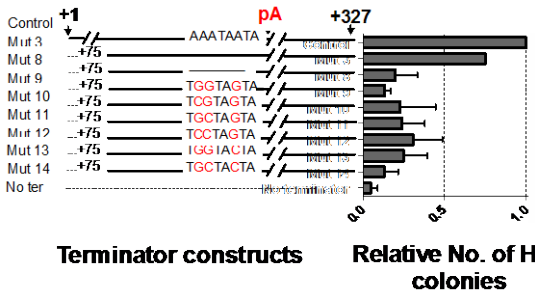

Figure 2. Mutational analysis of putative polyA signals in the *C. subvermispora gpd* terminator

in basidiomycetes. We conducted 1) determination of poly(A) sites of a reporter construct in stable *C. subvermispora* transformants and 2) mutational analysis of estimated poly(A) sites using a transfection system in *C. subvermispora* (Figure 2). Our results indicate that a well-conserved consensus sequence has a major function in polyadenylation and some complementary sequences with weak activity can also direct poly(A) addition to the 3' end of mRNAs.

Conclusions

Through transient reporter gene expression using a transfection system, we demonstrated: that an intron is not essential for efficient gene expression, arguing against an established theory. We also demonstrated that a transcriptional terminator can greatly influence gene expression levels and that, besides a strong poly(A) signal that is conserved among various eukaryotes, there is (an)other sequence(s) with weak polyadenylation activity in the selective lignin degrading fungus, *C. subvermispora*.

These results may foster the industrial utilization of basidiomycetes through the stable and efficient production of various recombinant proteins in the future. It should be noted that the best poster prize was awarded to the presentation of part of this research at the European Conference on Fungal Genetics, which was held in Haifa, Israel, in February 2018.

References

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