

# Translational enhancer that promotes protein biosynthesis and leads to enriched grain production

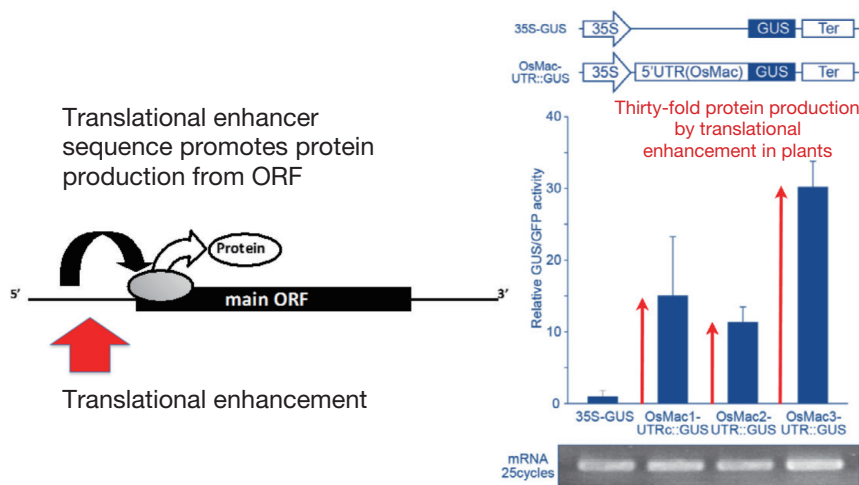
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## Purpose of Research

[POINT] Enhanced crop production by promoting protein biosynthesis from mRNA without increasing gene transcription. Most of conventional technologies to efficiently produce proteins do so by promoting mRNA production (gene transcription). Our technology is unique in that it depends on translational enhancers that promote protein synthesis from mRNA (translation). We have identified a novel translational enhancer derived from the *Oryza sativa* OsMac1, OsMac2, and OsMac3 genes. This enhancer produced a 5- to 30-fold increased amount of a reporter protein encoded by its downstream ORF. Currently we are working on the construction of a high-efficiency production system based on the function of such translational enhancers. The future goal is to establish a method applicable to highly efficient production of substances in various cell systems.

## Summary of Research

We investigated the usefulness of translational enhancers by testing the effect of one of them on the production of a reporter protein. The enhancer sequence was inserted in the 5'UTR that was located upstream of the coding region of a temperature-sensitive elastin-like peptide (ELP) fusion protein gene, to construct plasmids that expressed the reporter from a CaMV 35S promoter. These plasmids were introduced into *Nicotiana benthamiana* and *Oryza sativa*. Both of the resultant transformants showed a several fold increase in the amount of the ELP fusion reporter protein, compared to a control. This indicated that translational enhancers can promote translation in these plants.



## Comparison with Conventional or Competitive Technology

Conventional high-efficiency protein production technologies usually increase gene expression (transcription). The present technology is unique in that it enhances protein production from mRNA (translation). When combined with conventional technology, the present technology can greatly raise protein production through the synergistic effect of increased gene transcription and translation. It also enhances protein production in cell-free protein synthesis systems.

## Expected Applications

- Novel research reagents/kits (for high-efficiency protein production).
- Production of valuable proteins in a living plant.
- Research reagents for protein arrays using cell-free protein synthesis systems.

## Challenges in Implementation

- Research needed for practical applications, e.g. in nucleotide sequence optimization.

## What We Expect from Companies

Please contact us if you are interested in industrial application of the present technology.

## Points

- Our system yields increased protein production by promoting mRNA translation
- Our system may give a synergistic effect using a enriched transcription because it targets the translational process
- Our system also efficiently works on the protein production by cell-free protein synthesis system

## Future Developments

- Fiscal 2015: Application to various cell types
- Fiscal 2016: Research for practical applications (research reagents)

- Intellectual Property: Japanese Patent No. 5598899 “translational enhancer” Registered Japanese Patent Application No. 2014-102528 “translational enhancer” Application unpublished
- Reference: Hiromi Aoki, Hiroshi Teramura, Mikhail Schepetilnikov, Lyubov A Ryabova, Hiroaki Kusano, Hiroaki Shimada “Enhanced translation of the downstream ORF attributed to a long 5' untranslated region in the *OsMac1* gene family members, *OsMac2* and *OsMac3*” *Plant Biotechnology* Vol. 31 (2014) No. 3 p. 221-228  
Hiroshi Teramura, Yusuke Enomoto, Hiromi Aoki, Tadamasa Sasaki, Hiroaki Shimada  
A long 5' UTR of the rice *OsMac1* mRNA enabling the sufficient translation of the downstream ORF. *Plant Biotechnology* 29, 43-49 (2012)

