Characterization of a HRV3C protease-activated T7RNA polymerase (PA-RNAP) sensor system for studying protein-protein interaction *in E.coli*

Faying Zhang, Shengchen Wang, Shihui Yang, Guimin Zhang, Li Yi*

State Key Laboratory of Biocatalysis and Enzyme Engineering, Hubei Key Laboratory of Industrial Biotechnology, School of Life Sciences, Hubei University, Wuhan, 430062, China, ***liyi@hubu.edu.cn***

Abstract

Due to its high activity and specificity, T7 RNA polymerase (T7 RNAP) mediated transcription system has been proven to be simple and efficient for gene expression in *E. coli*, yeast, and human cells. Previous studies showed that T7 lysozyme could effectively inhibit the function of T7 RNAP. Based on this, a tobacco etch virus (TEV) protease-activated lysozyme-inhibiting T7RNA polymerase (PA-RNAP) system was further developed. In this system, the T7 RNAP mediated RNA transcription is inhibited by the T7 lysozyme, which could be activated by removing of lysozyme by using specific TEV protease. Despite of its exquisiteness, the PA-RNAP system has never been comprehensively characterized, and its effectiveness was limited by the mesothermal-adapted property and low activity of TEV protease[1]. Here, we use the human rhinovirus 3C (HRV 3C) protease to reconstitute this system, facilitating its effective utilization in a broad temperature range from 37 °C to 4 °C. Our comprehensive evaluation of the HRV 3C protease-activated lysozyme-inhibiting T7RNA polymerase system indicated that the lysozyme has to be anchored at the N-terminal of T7 RNAP with a flexible linker of 32 aa length to maximize its inhibition effects. Moreover, a new strategy for studying the protein-protein interaction under in vivo condition was developed, which was preliminarily applied for investigating the interactions of different subunits in the Type I-F Crispr-Cas complex of *Zymomonas mobilis*.

Introduction

T7 lysozyme is a natural inhibitor of T7RNAP. T7RNAP would temporarily become an inactive form when tethered with T7 lysozyme through a flexible linker containing a target protease cleavage site. The T7RNAp : T7 lysozyme complex then would be disassociated and fluorescence recovers when a specific protease cleave its cut site. Our characterization of this T7RNAp : T7 lysozyme system would further expand its applications on protein engineering and protein-protein interactions .

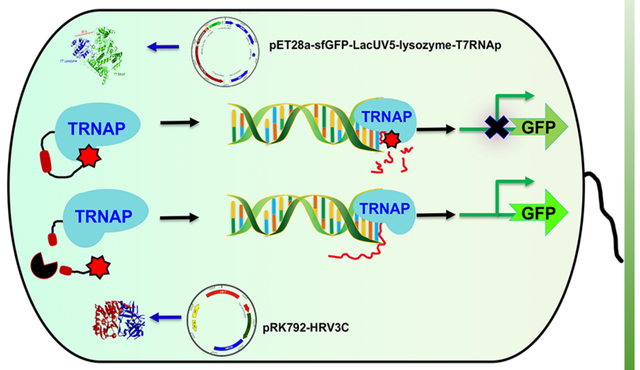

Figure 1: HRV3C protease-activated lysozyme-inhibiting T7RNA polymerase system

Results and discussion

I. Development of a HRV3C protease activated-RNAP system with a broader temperature range

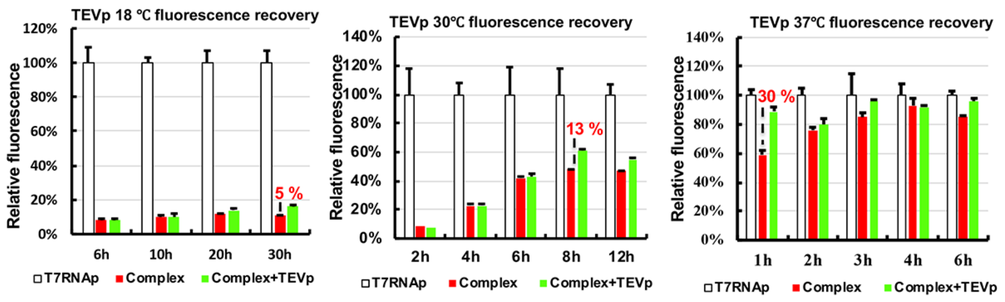

Figure 2: The comparing of TEV/HRV3C protease-activated lysozyme-inhibiting T7RNA polymerase system

The HRV3C protease activated-RNAP system displayed better inhibitive effect and fluorescence recovery ability in a broader temperature range comparing to TEV protease.

II. The comprehensive characterization and optimization of HRV3C protease activated-RNAP system

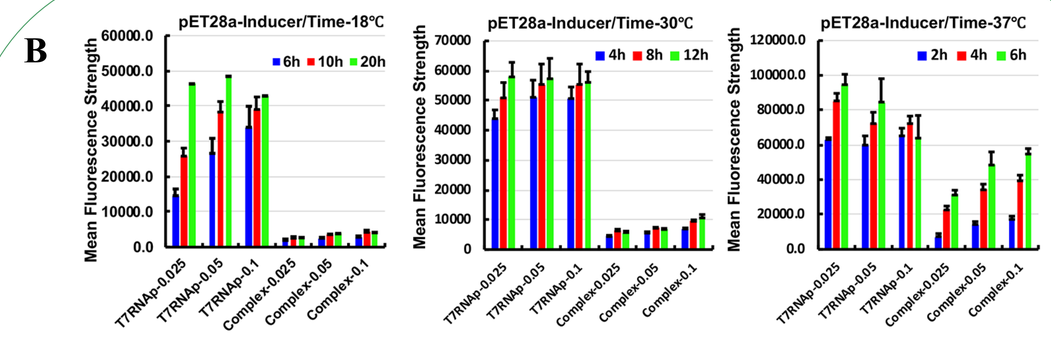

Figure 3: The characterization and optimization of HRV3Cp activated-RNAP system

A) The length of flexible linker between lysozyme and T7RNAP **B)** In vivo inducing condition.

III. Two strategies to further improve the fluorescence recovery ability

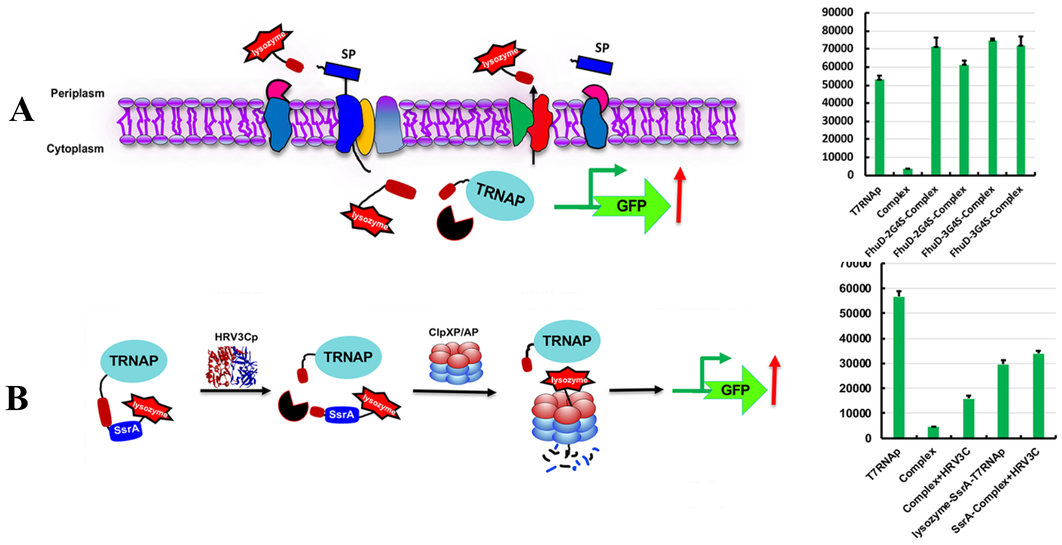

Figure 4: Two strategies to further improve the fluorescence recovery ability

Two strategies: A) secreting out of lysozyme with the help of signal peptides[2], B) degradation of lysozyme by SsrA-dependent degradation were designed to improve fluorescence recovery ability [3]. However, our results demonstrated that the adding of both the signal and SsrA tag components could obviously affect the inhibition effect and increase the undesirable fluorescence.

IV. The applications of a optimized HRV3C protease activated-RNAP system

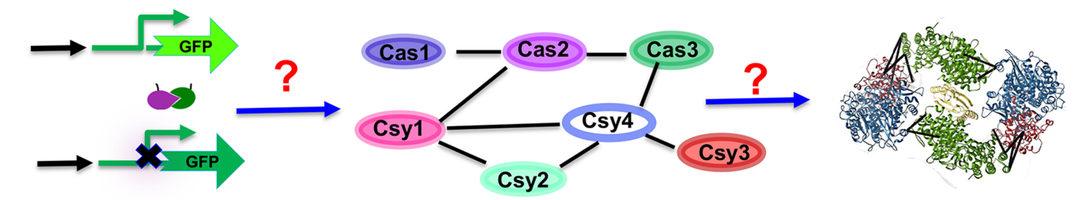

Figure 5: The preliminary application of an optimized HRV3Cp activated-RNAP system

The optimized system would be applied for investigating the interactions of different subunits in the Type I-F Crispr-Cas complex of *Zymomonas mobilis*[4].

Conclusion

- We have verified that HRV3C protease activated-RNAP system exhibited more efficient and better mesothermal-adapted property comparing to TEV protease.
- The HRV3C protease activated-RNAP system has been comprehensively characterized and optimized.
- In future work, the system would be further optimized for higher fluorescence recovery and be applied for investigating the interactions of different subunits in the Type I-F Crispr-Cas complex of *Zymomonas mobilis.*

Reference

[1] M.S. Packer, H.A. Rees, D.R. Liu, Phage-assisted continuous evolution of proteases with altered substrate specificity, *Nat Commun* 8(1) (2017) 956.

[2] R. Freudl, Signal peptides for recombinant protein secretion in bacterial expression systems, *Microb Cell Fact* 17 (2018). (52)

[3] C.M. Farrell , A.D. Grossman, R.T. Sauer, Cytoplasmic degradation of ssrA-tagged proteins, *Mol Microbiol* 57(6) (2015), 1750-61.

[4] Dong, G., He, M.X. and Feng, H. Functional Characterization of CRISPR-Cas System in the Ethanologenic Bacterium *Zymomonas mobilis* ZM4. *Adv Microbiol*, 6 (2016) , 178-189.