C-5-32

Theophylline-dependent Riboswitch as a Useful Genetic Tool for Synthetic Biology in Cyanobacteria

Yoichi Nakahira[1] nakahira.yoichi.mu@ehime-u.ac.jp
Atsushi Ogawa[2] a-ogawa@ccr.ehime-u.ac.jp
Hiroyuki Asano[3]
Tokitaka Oyama[3] oyama@cosmos.bot.kyoto-u.ac.jp
Yuzuru Tozawa[1, 2] tozawa.yuzuru.mx@ehime-u.ac.jp

[1] Venture Business Laboratory, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan
[2] Proteo-Science Center, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan
[3] Graduate School of Science, Kyoto University, Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan

Keywords: Synthetic biology, Theophylline riboswitch, Translational regulation, Cyanobacteria

Cyanobacteria are favorable cell factories for the production of renewable biofuels and valuable chemicals because of their ability to capture solar energy. However, biotechnology for cyanobacteria still lags behind conventional model species such as *Escherichia coli* or baker's yeast. In this work, we employed engineered riboswitches to control translational initiation of target genes in cyanobacterium *Synechococcus elongates* PCC 7942. A firefly luciferase reporter assay revealed that three theophylline riboswitches performed well in *Synechococcus*. Among the riboswitches, the best one exhibited very low leaky expression of luciferase and dose-dependent ON/OFF regulation of protein expression by theophylline. The maximum magnitude of the induction versus basal level was ~190-fold. We also adopted this riboswitch to another gene regulation system, in which expression of the circadian clock *kaiC* gene product is controlled in a theophylline dose-dependent manner. The result demonstrated that the adequately adjusted expression level of KaiC restored complete circadian rhythm in the *kaiC*-deficient arrhythmic mutant. These suggest that the theophylline-dependent riboswitch system has great promise as a useful genetic tool for synthetic biology in cyanobacteria.

[1] Nakahira, Y., Ogawa, A., Asano, H., Oyama, T., and Tozawa, Y., Theophylline-dependent riboswitch as a novel genetic Tool for strict regulation of protein expression in cyanobacterium *Synechococcus elongatus* PCC 7942, *Plant Cell Physiology*, in press, 2013.