Evolutionary design of gene expression systems

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Research aims

Various types of transcriptional controlling mechanisms have been investigated in the search for useful gene induction systems suitable for the microbial production of valuable chemicals. Although some of them, including arabinose-, lactose-, and tetracycline-inducible systems, perform well in the laboratory, they are not used in the large-scale commercial production of chemicals, simply due to high costs of inducer molecules. We aimed to develop a highly stringent, yet high-yield induction system using a safe and cheap induction system truly useable in real-world applications.

Methods

1. Improvement of the sensitivity of choline-inducing systems

Choline is a cheap and safe edible/nutrient compound found in egg yolk. We have previously conducted directed evolution of the BetI/BtO system, which is the choline induction system in *Escherichia coli*, into high-stringent and high power transcriptional induction systems (ver.1) [1) 2)]. These systems have been demonstrated as highly useful both in metabolic engineering and as tools for constructing genetic circuits. However, there remains one technical issue: it requires as much as 10 mM of choline to completely induce the genes.

We first conducted directed evolution of BetI in search for high-sensitive variants, but all our attempts were unsuccessful. Based on this failure, we concluded that choline import might be the bottle-neck. Therefore, we placed the choline transporter BetT under the control of Bet promoter (ver.2). The switching behavior of the resultant induction system was analyzed by transforming the *E. coli* strain (BW25113) harboring resultant systems where the fluorescent protein (sfGFP) is placed under P_{Bet}. The same experiment was conducted for a strain lacking *betA* (gene coding choline-metabolizing enzyme)

harboring the same plasmids.

2. Improving the switching property of Ptrp

Amino acids constitute other candidate molecules that can be safely used as inducers. Among these, tryptophan-responsive promoter systems (P_{trp}) have been widely used, including in the industrial production of insulin. For this system, the endogenous regulator TrpR is strong enough to act on the Trp promoter multi-copy plasmid, but non-negligible leakiness exists. Using a plasmid expressing TrpR, we could have drastically improved the stringency of the system, but drastic reduction of induction level was observed. We therefore tried to optimize the operator/promoter sequences to obtain higher induction levels while maintaining the stringency.

To this end, we scrambled the -10 sequence of the natural Trp promoter, which was significantly off from the consensus sequence of *E. coli* promoters. We also randomized one of the six TrpR-binding sequences (TrpOs), because TrpO located at the most upstream of TrpP was significantly off from the consensus sequence (GNACT).

The resultant TrpP variants library (containing five randomized nucleotides; 1,024 possible sequences) was inserted upstream of a plasmid encoding GFP, to search for those with elevated induction level and high-stringency.

Results

1. Reduction of choline inducers required by 3,500-fold increased sensitivity

When BetT was additionally expressed by placing the gene encoding betT under the control of Bet promoter, a drastic shift of transfer function of the BetI/BetO system was observed (Fig. 1). This new system (ver.2) only required less than 0.1 mM of choline to induce genes, which is 200 times less than that for the original system. Given the low price (ca. 5 USD/kg) of choline, this system requires 2 cents per liter of culture for inducer molecules. When installed into the BetA-deleted strains (ver.3), we observed another 20× reduction in the amount of choline required for induction, reaching down to ~5 μM. Thus, the inducer cost of the choline-inducible system becomes virtually negligible.

2. Ten times improvement in the Tryptophan induction system

Although the sequence diversity of the promoter library we designed was minimal (1,024), we could have isolated a variety of TrpP variants. Some of the obtained TrpP variants were found to show more than 10 times improvement in induction levels, while preserving stringency in un-induced conditions (Fig. 2).

Conclusion

Nature harbors countless genetic switches and all of them could be potentially useful as inducer systems for induction of enzymes/ pathways. However, it is rare that the specification of these switches is suitable for biotechnological uses without modification. To establish reliable induction systems without eliciting a physiological response in host organisms, the specifications of each natural genetic switch should be altered. This work demonstrated the surprising plasticity of switching properties of genetic switches. Using the directed evolution platform we have been developing[3) 4)], we should be able to rapidly provide a variety of genetic switches in each and every biotechnological contexts.

References

1) Ike K., et al.: Evolutionary design of choline- inducible and –repressible T7-based induction systems. ***ACS Synthetic Biology,*** 4, 1352-1360 (2015).
2) Saeki, K, et al.: Rapid diversification of BetI-based transcriptional switches for the control of biosynthetic pathways and genetic circuits. ***ACS Synthetic Biology,*** 5, 1201-1210 (2016).
3) Tominaga M, et al.: Rapid and liquid-based selection of genetic switches using nucleoside kinase fused with aminoglycoside phosphotransferase. ***PLOS One***, 10, e0120243, 1-11 (2015).
4) K. Ike and Umeno D: Nucleotide kinase-based selection system for genetic switches: ***Methods in Mol Biol***, 1111, 141-152 (2014).

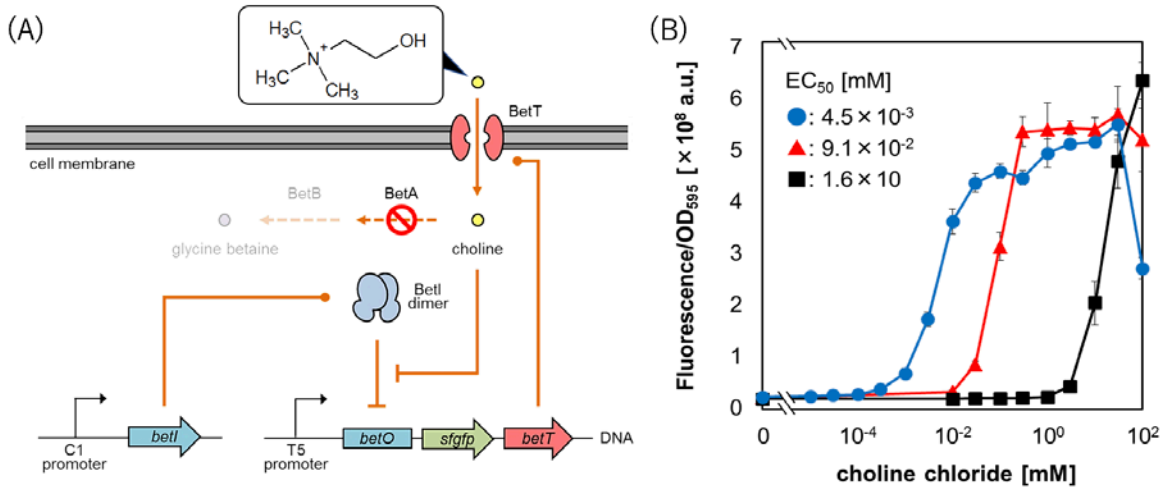

Figure. 1 Choline-inducible systems. (A) Plasmid expression system based on the Bet system. BetI repressed the transcription of T5 promoter fused with BetO,

and choline induces this promoter by binding and altering the structure of BetI, thereby departing from BetO. In Ver.2, the choline importer (BetT) is expressed under the control of T5P/BetO promoter, increasing the effective cellular concentration of choline. Choline concentration is also elevated by blocking its consumption (via deletion of BetAB). (B) Transfer function of Bet-systems. Ver.1 （■）, ver.2 （▲）, ver.3 （●）.

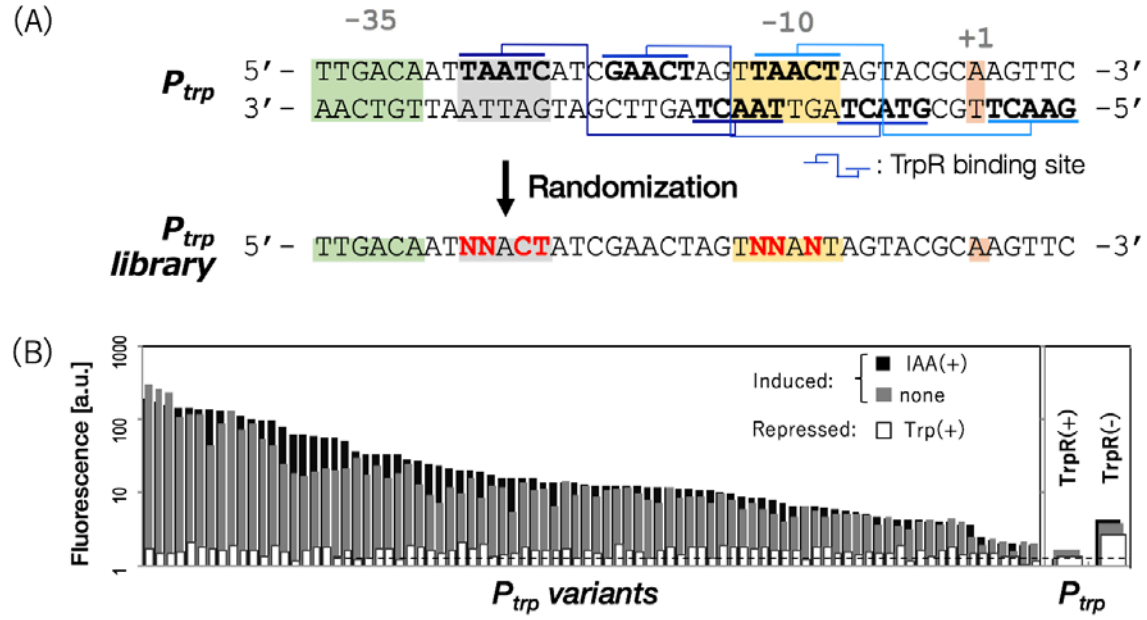

Figure. 2 A series of tryptophan induction systems. (A) Library design: three bases at -10 sequence and two bases at the first TrpO sequence were randomized, yielding 1,024 unique P_{Trp} variants. (B) Switching properties of TrpP variants.