Developing microbial cell factories by employing synthetic small regulatory RNAs

January 20 2013

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Biotechnologists have been working hard to address the climate change and limited fossil resource issues through the development of sustainable processes for the production of chemicals, fuels and materials from renewable non-food biomass. One promising sustainable technology is the use of microbial cell factories for the efficient production of desired chemicals and materials. When microorganisms are isolated from nature, the performance in producing our desired product is rather poor. Metabolic engineering is performed to improve the metabolic and cellular characteristics to achieve enhanced production of desired product at high yield and productivity. Since the performance of microbial cell factory is very important in lowering the overall production cost of the bioprocess, many different strategies and tools have been developed for the metabolic engineering of microorganisms.

One of the big challenges in metabolic engineering is to find the best platform organism and to find those genes to be engineered so as to maximize the production efficiency of the desired chemical. Even Escherichia coli, the most widely utilized simple microorganism, has thousands of genes, the expression of which is highly regulated and interconnected to finely control cellular and metabolic activities. Thus,

the complexity of cellular genetic interactions is beyond our intuition and thus it is very difficult to find effective target genes to engineer. Together with gene amplification strategy, gene knockout strategy has been an essential tool in metabolic engineering to redirect the pathway fluxes toward our desired product formation. However, experiment to engineer many genes can be rather difficult due to the time and effort required; for example, gene deletion experiment can take a few weeks depending on the microorganisms. Furthermore, as certain genes are essential or play important roles for the survival of a microorganism, gene knockout experiments cannot be performed. Even worse, there are many different microbial strains one can employ. There are more than 50 different *E. coli* strains that metabolic engineer can consider to use. Since gene knockout experiment is hard-coded (that is, one should repeat the gene knockout experiments for each strain), the result cannot be easily transferred from one strain to another.

A paper published in *Nature Biotechnology* online today addresses this issue and suggests a new strategy for identifying gene targets to be knocked out or knocked down through the use of synthetic small RNA. A Korean research team led by Distinguished Professor Sang Yup Lee at the Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), reported that synthetic small RNA can be employed for finely controlling the expression levels of multiple genes at the translation level. Already well-known for their systems metabolic engineering strategies, Professor Lee's team added one more strategy to efficiently develop microbial cell factories for the production of chemicals and materials.

Gene expression works like this: the hard-coded blueprint (DNA) is transcribed into messenger RNA (mRNA), and the coding information in mRNA is read to produce protein by ribosomes. Conventional genetic engineering approaches have often targeted modification of the blueprint itself (DNA) to alter organism's physiological characteristics. Again,

engineering the blueprint itself takes much time and effort, and in addition, the results obtained cannot be transferred to another organism without repeating the whole set of experiments. This is why Professor Lee and his colleagues aimed at controlling the gene expression level at the translation stage through the use of synthetic small RNA. They created novel RNAs that can regulate the translation of multiple messenger RNAs (mRNA), and consequently varying the expression levels of multiple genes at the same time. Briefly, synthetic regulatory RNAs interrupt gene expression process from DNA to protein by destroying the messenger RNAs to different yet controllable extents. The advantages of taking this strategy of employing synthetic small regulatory RNAs include simple, easy and high-throughput identification of gene knockout or knockdown targets, fine control of gene expression levels, transferability to many different host strains, and possibility of identifying those gene targets that are essential.

As proof-of-concept demonstration of the usefulness of this strategy, Professor Lee and his colleagues applied it to develop engineered *E. coli* strains capable of producing an aromatic amino acid tyrosine, which is used for stress symptom relief, food supplements, and precursor for many drugs. They examined a large number of genes in multiple *E. coli* strains, and developed a highly efficient tyrosine producer. Also, they were able to show that this strategy can be employed to an already metabolically engineered *E. coli* strain for further improvement by demonstrating the development of highly efficient producer of cadaverine, an important platform chemical for nylon in the chemical industry.

This new strategy, being simple yet very powerful for systems metabolic engineering, is thus expected to facilitate the efficient development of microbial cell factories capable of producing chemicals, fuels and materials from renewable biomass.

More information: Dokyun Na, Seung Min Yoo, Hannah Chung, Hyegwon Park, Jin Hwan Park, and Sang Yup Lee, "Metabolic engineering of Escherichia coli using synthetic small regulatory RNAs", *Nature Biotechnology*, doi:10.1038/nbt.2461 (2013)