FUNDING PROGRAM FOR NEXT GENERATION WORLD-LEADING RESEARCHERS

Project Title: Genetic indoctrination of yeast for innovative drug discovery	
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1. Background of research

Polyketides (PKs) and nonribosomal peptides (NRP) have been isolated from *Streptomyces* and many other source organisms. In recent years, biosynthetic gene clusters encoding PK synthases (PKSs) and NRP synthetases (NRPSs) have been discovered in fungal genome, fully sequenced, and published in scientific periodicals. On average, fifty biosynthetic genes have been identified on a genome of a single fungus. However, fewer fungal PKs and NRPs have been identified than we have anticipated under standard growth conditions. A fungal heterologous expression system would prove inferior and inadequate to accommodate the many genes contained in a PKS and NRPS gene cluster. Additionally, to heterologously express fungal biosynthetic genes in the range of 5 to 20 kb, the transcriptional system requires splicing for functional biosynthetic proteins due to the inherent difficulty in predicting the intron region required for extraction by PCR. Our preliminary results clearly demonstrate the successful expression of a fungal PKS gene from *Chaetomium globosum* genome in a yeast expression system. The yeast system using *Saccharomyces cerevisiae* clearly provides an advantage over a fungal system in terms of molecular cloning and its ability to tolerate substantially large genes. This system is much easier and faster when constructing an expression plasmid by recombination cloning for reconstitution of biosynthetic gene pathway. The plasmid-borne biosynthetic pathway makes it possible to synthesize many analogs through traditional molecular biological techniques.

2. Research objectives

The proposed goal of our project is to establish an innovative methodology for biosynthesizing bioactive molecules by administering gene expression in yeast. This will facilitate efforts in isolating novel natural products, rational engineering of proteins involved in biosynthesis of these products and the ability to generate analogs possessing comparable if not more potent bioactivity.

3. Research characteristics (incl. originality and creativity)

The studies described in this proposal constitute essential steps toward a long range goal of increasing the availability of secondary metabolites for pharmaceutical development by taking advantage of some innovative engineering strategies developed in our laboratory. Application of such technology will enhance endeavors of obtaining elusive natural products by transplanting their biosynthetic gene clusters to a more manageable host such as yeast. Moreover, it will be more effectual to synthesize conceptualized analogs at a genetic level by introducing modifications to the biosynthetic pathway as a plasmid-borne system using yeast as a homologue recombinant cloning host. These studies will fully utilize our background in chemical synthesis, natural product chemistry and systematic engineering of biosynthetic enzymes and pathways.

4. Anticipated effects and future applications of research

Expression of the genome encoding PK and NRP biosynthetic proteins found in fungi has proven to be a powerful tool by enabling scientists to successfully generate novel molecules for drug discovery.