【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Agricultural sciences)

# Title of Project : Chemical Genetics on Novel Function of Splicing Factors

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Research Area : Agriculture

Keyword : Splicing, Intron, Transcription, Nucleo-cytoplasmic transport, non-coding RNA

## 【Purpose and Background of the Research】

Pre-mRNA transcribed from DNA in eukaryotes including humans contains intervening sequence called introns, having no amino acid sequence information. Splicing, which eliminates introns from pre-mRNA, is essential for proper gene expression and variation of the splicing pattern contributed to evolution. On the other hand, a novel "RNA continent" consisting of a vast number of non-coding RNA (ncRNA) has recently been discovered, and functional ncRNAs such as miRNA or snoRNA have also been found out in introns, which had been thought to be meaningless sequences. However, functions of introns have yet uncharacterized, because there was no specific inhibitor of splicing, which allows to control the splicing reaction. Our studies on the mode of action of spliceostatin A (SSA) revealed that SSA inhibits splicing by binding to a spliceosome component SF3b. SSA treatment allowed translation of a subset of pre-mNRA, affected localization and stability of nuclear ncRNAs, and altered the chromatin modification status, which suggests that SF3b is involved in not only splicing but also other multiple biological functions. This project aims to elucidate the novel functions of splicing factors and introns by the chemical biology using SSA.

## 【Research Methods】

To understand the function of the splicing factor SF3b, the project should be focused on following four points, (1) the role in mRNA quality control, (2) the role in regulation of transcription and chromatin, (3) the role in ncRNA control, and (4) the role in structure and function of nucleoli and nuclear domains. (1) We will analyze the mechanism by which SF3b retain pre-mRNA in the nucleus and what happens in the complex when SSA targets the spliceosomes containing SF3b. (2) SSA induces transcriptional activation of some genes, while many other genes vice versa are repressed. In addition, global change of modification status in histones was observed. We will analyze how SF3b is involved in global transcription and chromatin regulation. (3) We have observed that SSA caused drastic changes in the localization or the cellular amount of several nuclear ncRNA such as Gomafu and Xist. The mechanism of how splicing factors such as SF3b regulate ncRNA metabolism will be analyzed. (4) SSA caused changes in the morphology of SC35 nuclear speckles in mammalian cells and nucleolus in fission yeast cells. Taking advantage of our ORFeome cloning of the fission yeast in genomic genes, we will globally analyze what gene products are involved in the morphological changes in the nuclear structures.

## 【Expected Research Achievements and Scientific Significance】

SSA is the first small molecule inhibitor of splicing causing translation of pre-mRNAs of some genes such as the p27 CDK inhibitor, which implies that nuclear retention of pre-mRNA is simultaneously blocked by SSA. Thus it seems possible that the unknown mechanisms underlying the pre-mRNA nuclear retention will be elucidate by this study. In addition, we expect that novel roles of splicing factors or introns will be unveiled by analyzing other SSA phenotypes including changes in gene expression and chromatin structure, ncRNA metabolism, and nuclear domains.

## 【Publications Relevant to the Project】

・Kaida et al. *Nature Chem. Biol.* 3: 576-583, 2007
・Lo et al. *Biochem. Biophys. Res. Commun.*, 364: 573-577, 2007
・Matsuyama et al. *Nature Biotechnol.* 24: 841-847, 2006

【Term of Project】 FY2009-2013

【Budget Allocation】 153,700 Thousand Yen

## 【Homepage Address and Other Contact Information】

http://www.riken.go.jp/r-world/research/lab/wako/chemi-gene/index.html
http://www.rikenresearch.riken.jp/frontline/573/