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SUMMARY of
2015 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Crystallization of Microprocessor component
Applicant	**Name**	**Soo Jae Lee**
	Affiliation	**College of Pharmacy, Chungbuk National University, Korea**
	Present Title	**Professor**
Research Collaborator (Host PI)		**Atsushi Nakagawa**

Summary

A number of different proteins are involved in miRNA processing. miRNAs are first processed in the nucleus. The pri-miRNA produced by Pol II is cleaved at the stem of the hairpin structure, which releases an approximately 60–70 nt hairpin structure, known as the precursor miRNA (pre-miRNA). This processing step is performed by Drosha, which requires the DiGeorge syndrome critical region in gene 8 (DGCR8) in humans as a cofactor. Drosha, in conjunction with DGCR8 forms a large complex known as the microprocessor components. DGCR8 and Drosha are largely conserved in animals. Typically, pri-miRNAs are comprised of about 33 base pairs of the stem loop and a terminal loop and single-strand RNA flanking segments. DGCR8 interacts with RNA segment and guides Drosha to slice pri-miRNA. Drosha cleaves RNA duplexes about 11 bp away from the ssRNA-stem loop junction and thus processes the pri-miRNA to the pre-miRNA with a 5′-phosphate group and an approximately 2 nt 3′ overhang.

In this study, we tried to crystallize the microprocessor components. For crystallization of proteins Drosha/DGCR8 and pri-miRNA complex, the most urgent step is the overexpression of proteins. The preliminary overexpression study result of the Drosha with E. coli. at our lab was a disappointing one. Prof. Nakagawa lab in IPR has many experience for expression of protein with baculovirus expression system. At the first stage, Drosha-DGCR8 co-expression by baculovirus was tested by IPR group, doing side by side RNA synthesis by Chungbuk Nat'l Univ. The complex crystallization was on going at Chungbuk Nat'l Univ. Although the final destination to this project is the structural and functional analysis of Drosha/DGCR8/pri-miRNA complex, as a starting project, the crystallization of DGCR8/ pri-miRNA complex was also tried. For crystallization of DGCR8 deletion mutant and pri-miRNA complex, we mixed the protein and RNA in a various stoichiometric ratios. The initial screening was performed using a PEG screen kit designed in our laboratory. A plate type crystal was appeared in two days and grows up to a week in PEG 3350 based condition. We could get some plate type crystal was appeared in two days and grows up to a week in PEG 3350 based condition. X-ray data sets were collected on the BL44XU beamline at Spring-8. The final improved crystal was diffracted to 3.4 angstrom resolution. However the phase determination was difficult owing to the imperfection of crystal.