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**SUMMARY of
2016 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University**

Research Title		Crystallization of Microprocessor component
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Summary		
<p>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.</p> <p>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. In this study, we got crystals of the RNA complex of the microprocessor component. For the crystallization of proteins DGCR8 and pri-miRNA complex, we expressed and purified the DGCR8 deletion mutants and mixed the protein with RNA in a various stoichiometric ratio. The initial screening was performed using a PEG screen kit designed in our laboratory. A plate type crystal was appeared in two days and grew 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. Recently, we could get another type crystals. It showed a weak diffraction pattern. Although the diffraction was weak, the crystal showed relatively low mosaicity.</p>		