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

Title of Project : Elucidation of molecular mechanisms of how piRNAs maintain the germline genome integrity from invasive mobile elements

Mikiko Siomi
(The University of Tokyo, Graduate School of Science, Professor)

Research Area : Biology
Keyword : PIWI, piRNA, transposon, RNA silencing, Drosophila

【Purpose and Background of the Research】

The germ cells have specific chromatin organization that enables them to express germline-specific genes. However, this permits the amplification and insertion of transposable elements, including transposons, into other sites in the genome, leading to injury in the genome, defects in gametogenesis and finally infertility. To avoid this, piRNA-mediated RNA silencing represses the activity of transposable elements. piRNAs are mainly derived from transposable elements and loaded onto PIWI proteins to form piRISCs. Both piRNAs and PIWI proteins are necessary for repressing transposonable elements in the germline. However, the molecular mechanisms of piRNA-mediated RNA silencing remain elusive. In this project, we will pursue our study to understand the molecular mechanisms by mainly focusing on piRNA biogenesis and their nuclear silencing functions.

【Research Methods】

We will use biochemical approaches to understand germline-specific RNA silencing pathways at the molecular level. We will also use new techniques related to deep-sequencing,

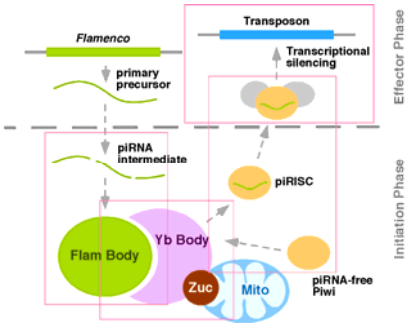

Figure: Model of OSC-piRNA biogenesis and function

bioinformatics and immuno-EM. Our first goal is to understand the molecular mechanism of piRNA biogenesis. We will focus on individually primary processing pathways and amplification loop. The question of how the piRNA machinery recognizes piRNA precursors over other RNA molecules in cells is very important to be addressed. Our second goal is to identify the molecular functions of piRNA factors. The targeted goal of this proposal is to use biochemical-based techniques to understand the mechanisms controlling germline cell synthesis and function with a view to biotechnological and therapeutic applications.

【Expected Research Achievements and Scientific Significance】

We aim to use a unique biochemical-based aspect to understand the role of RNA silencing in germ cell fate determination, maintenance, and differentiation. Molecular investigations have been carried out using ovaries and testes. However, these materials are limited and resources such as cell lines comprising only germ cells or somatic cells from ovaries or testes are indispensable. Our group has established OSC line from the parental fGS/OSS line containing both germline stem cells and ovarian somatic cell sheets. Also, we have the ability to carry out RNAi-based gene screening in the OSC line, monoclonal antibody production, immunoprecipitation, and small RNA library construction. Our expertise allowed us to biochemically analyze the functions of piRNA factors in the piRNA pathways, and gain new insights into the molecular function of Piwi,

【Publications Relevant to the Project】

・Saito K, Inagaki S, Mituyama T, Kawamura Y, Ono Y, Sakota E, Kotani H, Asai K, Siomi H, Siomi MC. A regulatory circuit for piwi by the large Maf gene traffic jam in Drosophila. ***Nature*** 461: 1296-1301. 2009
・Nishimasu H, Ishizu H, Saito K, Fukuhara S, Kamatani MK, Bonnefond L, Matsumoto N, Nishizawa T, Nakanaga K, Aoki J, Ishitani R, Siomi H, Siomi MC*, Nureki O*. Structure and function of Zucchini endonuclease in piRNA biogenesis. ***Nature*** 491: 284-289. 2012 (*double corresponding)

【Term of Project】 FY2013-2017

【Budget Allocation】 160,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://www-siomilab.biochem.s.u-tokyo.ac.jp/index.html