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

Broad Section G

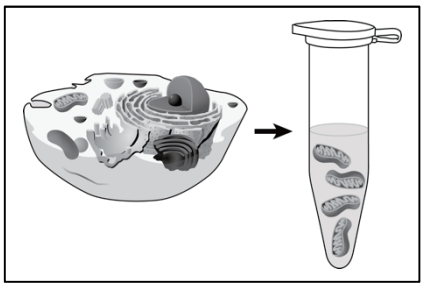
Title of Project : Biochemical approaches to understanding the reaction platforms of the piRNA pathway

Yukihide Tomari
(The University of Tokyo, Institute for Quantitative Biosciences, Professor)

Research Project Number : 18H05271 Researcher Number : 90447368
Keyword : piRNAs, small RNAs, reaction platform, RNA silencing, PIWI, Argonaute

【Purpose and Background of the Research】

Since the discovery of RNA interference, our biochemical understanding of small RNAs has been greatly advanced. However, there remain many unknowns in the molecular mechanism of piRNAs, which play essential roles in protecting the germline cells from transposons. The biggest obstacle is that piRNAs require specific "reaction platforms" in cells for their biogenesis and function, and thus the natural activity can be easily lost in conventional biochemical approaches using soluble lysates prepared by high-speed centrifugation, purified recombinant proteins etc.
We have previously established a cell-free system that recapitulates a part of the piRNA biogenesis pathway using the whole mitochondrial fraction. In this project, we propose to further develop this unique in vitro system so as to precisely understand the biogenesis and function of the piRNA pathway at the molecular level.



Biochemical approaches to understanding the reaction platforms

【Research Methods】

In particular, we will focus on the following three questions.

1. How are piRNA intermediates loaded into PIWI proteins?
2. How are piRNA intermediates processed into mature piRNAs?
3. How are the piRNA-cleaved targets are properly handed over to the next PIWI protein?

We seek to extract the corresponding "reaction platforms" from cells to test tubes in their best intact forms, faithfully recapitulate the processes and monitor the on-site reactions. We will not only utilize biochemistry to dissect the reactions into fundamental steps, but also combine it with genome editing technologies, next-generation sequencing, bioinformatics etc.

【Expected Research Achievements and Scientific Significance】

Our molecular understanding of the piRNA pathway is still lacking in vague models. This project takes advantage of our unique knowledge and approaches to the characteristic features of piRNAs that depend on cellular "reaction platforms" and aims at breaking the deadlock in the field. The idea of "reaction platform"-focused biochemistry is not limited to the piRNA pathway but could also be applicable to various non-coding RNAs and other biological processes that relies on cellular platforms.

【Publications Relevant to the Project】

Structural basis for arginine methylation-independent recognition of PIWIL1 by TDRD2. Zhang H, Liu K, Izumi N, Huang H, Ding D, Ni Z, Sidhu SS, Chen C, *Tomari Y, *Min J. ***Proc Natl Acad Sci U S A.*** 2017 Nov 21;114(47):12483-12488.

Identification and functional analysis of the pre-piRNA 3′ Trimmer in silkworms. Izumi N, Shoji K, Sakaguchi Y, Honda S, Kirino Y, Suzuki T, Katsuma S, *Tomari Y. ***Cell.*** 2016 Feb 25;164(5):962-73.

3′-end formation of PIWI-interacting RNAs in vitro. Kawaoka S, Izumi N, *Katsuma S, *Tomari Y. ***Mol Cell.*** 2011 Sep 16;43(6):1015-22.

【Term of Project】 FY2018–2022

【Budget Allocation】 148,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.iam.u-tokyo.ac.jp/tomari/
tomari@iam.u-tokyo.ac.jp