CDB SEMINAR

Speaker: Yukihide Tomari

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Title: "The putative RNA helicase Armitage is required for RISC assembly in *Drosophila* RNAi"

Date:	Monday, June 7
Time:	16:00 P.M.～ 17:30 P.M.
Place:	7th floor Conference Room of Building A, CDB

Summary:

In eukaryotes, long double-stranded RNA (dsRNA) silences genes homologous in sequence, a process termed RNA interference (RNAi). RNAi defends the genome against mobile genetic elements, such as transposons and viruses, whose expression and activity increase in RNAi-defective mutants. Long dsRNA is converted by Dicer into small interfering RNAs (siRNAs), which serve as the specificity determinants of the RNAi pathway. siRNAs direct mRNA cleavage as part of a protein-siRNA complex called the RNA-induced silencing complex (RISC). Several RISC components are known, and many of which are required for plant or animal development, implying that RNAi and/or miRNA-directed regulation functions in development.

Genetic studies reveal the importance of helicase-domain proteins in the RNAi pathway. Putative DEA(H/D)-box helicases are required for posttranscriptional gene silencing (PTGS) in the *Chlamydomonas reinhardtii* and RNAi in *C. elegans*. In *Drosophila*, mutations in *spindle-E* (*spn-E*), a gene encoding a putative DEAD-box helicase, abrogate endogenous RNAi-based repression of the *Stellate* locus and trigger expression of retrotransposon mRNA in the germline. In cultured *Drosophila* S2 cells, the putative helicase Dmp68 is a component of affinity-purified RISC. Similarly, a putative DEAD-box RNA helicase, Gemin3, is a component of human RISC. Dicer, too, contains a putative ATP-dependent RNA helicase domain. Except for Dicer, no specific biochemical function in RNAi has been ascribed to any of these helicase proteins.

armitage (*armi*) was identified in a screen for maternal effect mutants that disrupt axis specification in *Drosophila*. Armitage protein (Armi) is a member of a family of putative ATP-dependent helicases distinct from the DEA(H/D) box proteins. Armi is homologous across its putative helicase domain to SDE3, which is required for PTGS in *Arabidopsis*. Here, we show that *armi* is required for RNAi. *armi* mutant male germ cells fail to silence *Stellate*, and lysates from *armi* mutant ovaries are defective for RNAi in vitro. Native gel analysis of protein-siRNA complexes revealed at least three distinct complexes (B, A and R) in the assembly pathway of RISC. We show that complex B and A are the precursors of complex R (mature RISC) and that complex A contains the previously identified

R2D2/Dcr-2 heterodimer. Our data suggest that *armi* is dispensable for assembly of the early complexes B and A, but is required to convert complex A into mature RISC.

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