Molecular genetics of RNA interference and epigenetic gene silencing in a unicellular green alga *Chlamydomonas*

Project Leader

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Project Outline

In eukaryotes, genes in heterochromatic regions are transcriptionally silent because RNA polymerase is not accessible to the promoter. Histone modifications in the heterochromatic regions are distinguished from those in euchromatin. Moreover, dynamic conversion of a region from euchromatin to heterochromatin, or vice versa, is essential for the normal cell cycle to survive under stress.

RNA interference (RNAi) is originally identified as a system that degrades a specific mRNA that contains a sequence complementary to the siRNAs generated from transposon coding region, mating type locus, inverter repeat locus, direct repeat locus, etc. Recent investigations revealed that siRNA could be a trigger to nucleate heterochromatin. The heterochromatic state of a specific region can be carried over even after meiotic cell division. Methylated-CpG seems essential for long-term maintenance of heterochromatic state.

So far, investigation on the molecular mechanism of RNAi have mainly been carried out in yeast, S*accharomyces pombe,* because of its simple gene organization and the sophisticated genetic tools and rich resources. However, comparative analyses of RNAi systems in distantly related organisms are essential to elution of their universal cores. This is the why we recently started analyses of RNAi systems and their related epigenetic silencing in a unicellular green alga *Chlamydomonas reinhardtii.*

1. Method for isolating novel genes related to RNAi and epigenetic silencing

We have succeeded in artificially inducing RNAi by inducing a transgene that produces a hairpin RNA bearing potency to degrade the mRNA for spectinomycin resistance. We are isolating novel RNAi related genes through a series of random

destructions of endogenous genes by the tagging-method, identification of ~~the~~ tag inserted genes, and complementation of the wild type gene to rescue the phenotype.

2. Characterization of the function of RNAi related genes

Rapidly accumulating data show close relationships among DNA methylation, histone modifications, and epigenetic silencing. The function of RNAi related genes is analyzed through various methods including Northern hybridization for the siRNA and the target mRNA, genome bisulfite PCR for DNA methylation and ChIP for histone modifications. Furthermore, pull-down analyses of the relevant protein-containing complex will be carried out.

References

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