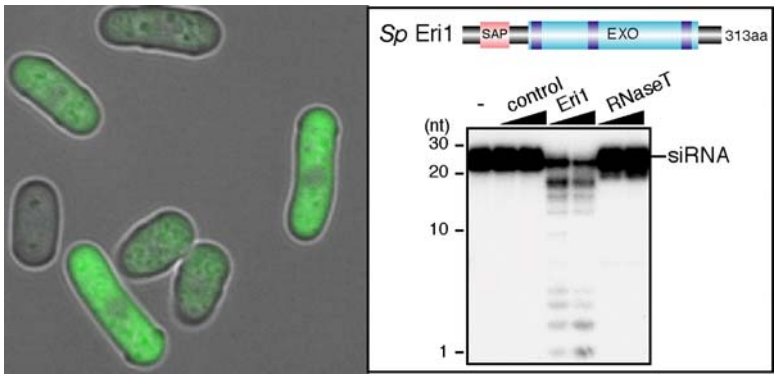
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

New player in RNAi-mediated gene silencing: Eri1 downregulates heterochromatin assembly

June 23, 2006 – Heterochromatin, a configuration of DNA and proteins coiled upon itself into highly condensed bundles, packs so tightly that it frequently serves to keep gene-encoding (euchromatic) regions out of the reach of the nuclear transcription apparatus, strongly preventing the genes so packaged from being expressed. The structure and role of such heterochromatic regions has been widely studied in organisms from yeast to human, and it is now known to have important functions in epigenetic regulation and the control of gene expression during cell differentiation as well. RNA interference (or RNAi) represents a second means by which the expression of genes can be blocked, by causing the degradation of short stretches of RNA prior to their translation into proteins. Interestingly, recent studies have shown that the RNAi machinery is involved in heterochromatin assembly, but the details of this mechanism remain tantalizingly obscure.

Now, work by Tetsushi Iida of the Laboratory for Chromatin Dynamics (Jun-Ichi Nakayama; Team Leader), shows that, in the fission yeast *Schizosaccharomyces pombe*, the ribonuclease Eri1 breaks down small interference RNAs derived from heterochromatic regions, thereby controlling heterochromatin assembly. These findings are of particular interest, as the molecular players involved are widely conserved across the biological spectrum, including in man. The results of the Nakayama team's study were published in the June 22 online edition of *Current Biology*.



Eri1 expressed in fission yeast cells (Left). Schematic diagram of Eri1 protein (Right top). Eri1 degraded double-stranded siRNA to the level of short oligonucleotides (Right bottom).

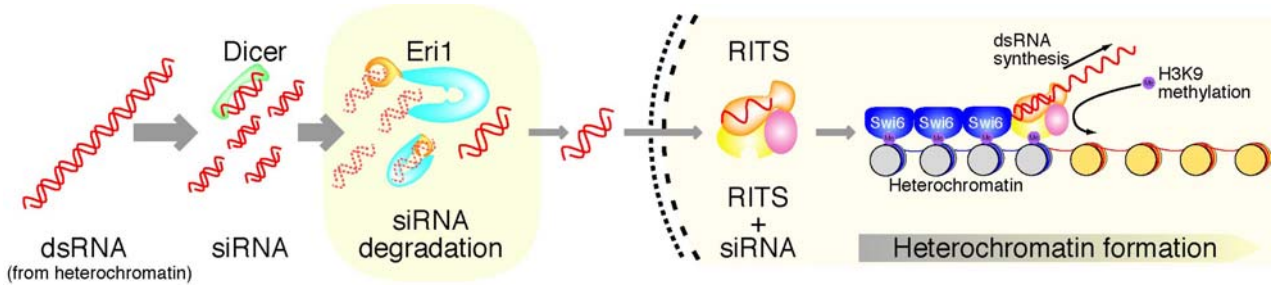
Eri1 was first discovered in the nematode, *C. elegans*, and this ribonuclease has since been found to have orthologs in a broad range of genomes. It is known to play a regulatory role in the RNAi machinery, in which short interfering double stranded RNA pairs up with complementary strands of messenger RNA, marking them for degradation. This RNAi mechanism is also believed to contribute to heterochromatin assembly. In this model, a bloc of proteins called the RITS (for RNA induced transcriptional silencing) complex ushers siRNAs to complementary chromosomal DNA, and thus initiate heterochromatinization.

Iida et al began by identifying the *S. pombe* ortholog of Eri1, and verifying that it too had ribonuclease activity against siRNAs in vitro. Zeroing in on a possible role in

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs

heterochromatin formation, they engineered mutant yeast cells that lacked *eri1*, and found that centromeric and other heterochromatic regional gene expression was repressed, apparently due to the absence of Eri1 enzymatic activity. They next showed that Eri1's function is linked to the RNAi machinery, which was already known to control the heterochromaticity of these chromosomal precincts. From this evidence, they surmised that Eri1 has a negative regulatory effect on heterochromatic gene silencing, by targeting and breaking down specific siRNAs.

In *S. pombe*, heterochromatin is defined by the highly specific protein modifications (the methylation of histone H3 at lysine 9), which, thus marked, becomes a target for the factor Swi6, leading to the tight winding for the affected sequence into a gene-silencing heterochromatic coil. In *eri1* mutants, histone methylation and Swi6 were enriched, and heterochromatin assembly at the centromeres upregulated. These same mutants also saw an increase in the abundance of siRNAs generated by the RNAi machinery, as evidenced by their increased association with the RITS complex, which suggests that eri1 normally functions to downregulate the buildup of siRNAs and their binding to RITS complexes.



Eri1 negatively regulates heterochromatin formation by degrading siRNA molecules; with the RITS complex activated by association with siRNAs, the chromosome region having sequence homology to the siRNAs subsequently undergoes heterochromatin formation. Eri1 degrades siRNA and is thought to regulate heterochromatin formation at an appropriate level.

The findings from these experiments led the Nakayama team to develop a model of heterochromatin assembly in fission yeast in which Eri1 enzymatically attacks heterochromatic region-derived siRNAs which would otherwise activate the RITS complex to drive further heterochromatin assembly, creating a perfectly titrated set of molecular checks and balances that keeps the cell's gene expression in tune. "It looks like Eri1 pulls off the neat trick of ensuring that heterochromatin assembles only where it should by maintaining siRNAs at just the right level," says Nakayama. "We've already begun to see from work in other labs how Eri1 can affect siRNA accumulation in *C. elegans* under certain conditions, so it will be exciting to find out more about its range of functions in RNAi."

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs