Chromodomain Proteins, Hrp1 and Hrp3, which are Required for Heterochromatin Silencing in Schizosaccharomyces pombe

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Hrp1 and Hrp3 are members of the CHD protein family of *Schizosaccharomyces pombe*. CHD proteins are thought to be required for modification of the chromatin structure in transcription, but the exact roles are not known. In this study, the effects of Hrp1 and Hrp3 on heterochromatin silencing were examined using the *S. pombe* strains containing marker gene in the highly transcriptional repressed regions such as centromere, telomere, mating type locus, and rDNA. Hrp1 was found to be involved in the transcriptional repression in the heterochromatin region such as centromere, mating type locus and rDNA repeats. ChIP assay showed that Hrp1 interacted to mating type locus directly. An *S. pombe* homologue of *hrp1*$^+$, named *hrp3*$^+$, was identified and found to be a non-essential gene. Silencing effect was also examined using the strains that contained marker gene in heterochromatin regions. *hrp3* deletion mutant alleviated the repression of silencing regions such as centromere, mating type locus, telomere and rDNA repeats. These results showed that Hrp1 and Hrp3, CHD1 proteins, are related with heterochromatin silencing and play a role as chromatin remodeling factors in vivo.

F114 Characterization of Chromodomain Proteins, Hrp1 and Hrp3, which are Required for Heterochromatin Silencing in Schizosaccharomyces pombe

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F115 Cloning, Expression and Characterization of NIP2, A Novel Nek2 Interacting Protein

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Nek2 is a mammalian ser/thr kinase that is closely related to *Aspergillus* NIMA. The structural homology between Nek2 and nimA suggests that, like NIMA, Nek2 is involved in the cell cycle regulation. From yeast two hybrid screening Nek2 as a bait, we cloned a novel gene named as NIP2 (Nek2-Interacting-Protein 2). Northern blot hybridization analysis using human tissue

blot showed ubiquitous expression of NIP2 mRNA but predominantly in the testis.Immunofluorescence staining indicated that NIP2 was localized to centrosomes and mitotic spindle pole during mitosis. Furthermore, Nek2 directly phosphorylated NIP2 bacterially purified as well as immunoprecipitated from cell lysates in vitro. These results suggest the functional relationship between Nek2 and NIP2.

F116 Functional Interaction between Telomere-associated Protein Taz1 and Rap1 homolog and their Roles at Telomeres of Schizosaccharomyces pombe

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Taz1 and TRF1/TRF2 were identified as telomeric repeat binding proteins from the fission yeast and mammalian cells, respectively. Recent report demonstrated that human Rap1(hRap1) is recruited to telomere by interaction with TRF2, arguing different control mechanisms of telomere between the budding yeast and mammal. Taz1 has no sequence homology with Rap1 but showed significant similarity to human TRF2, suggesting that they share common features in telomere regulation. Taz1 is implicated in multiple cellular functions. To assess the roles of Taz1 in telomere-related functions in detail, we attempted to identify the proteins interacting with Taz1 by using two-hybrid screening. Interestingly, sequence analysis of the insert cDNA from the positive clone revealed to have perfect matches with Rap1 homolog from S. pombe(spRap1), showing a significant homology with scRap1 and hRap1. The result might support our speculation that telomere regulation in the fission yeast is simmilar to that of higher eukaryotic cells. At present, we are investigating whether the localization of spRap1 at telomeres depends on its interaction with Taz1, and the role of spRap1 in the regulation of telomere function by analysis of knock-out mutant.

F117 Two Ubiquitin-Conjugating Enzymes, Rhp6 and Ups1 Regulate Silencing in Fission Yeast

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In *Schizosaccharomyces pombe*, centromeres, telomeres and silent mating type loci are subject to position effect control, called heterochromatic silencing. To understand the molecular basis of this phenomenon, genetic screen was performed to identify genes which, when overexpressed, disrupted silencing of mat3 locus. Two genes were isolated and found to encode Rhp6, a S. pombe homolog of Saccharomyces cerevisiae Ubc2/Rad6, and a novel putative ubiquitin-conjugating enzyme named Ups1 (Ubiquitin-conjugating enzyme participating in silencing). Overexpression of rhp6+ or ups1+ also disrupted the silencing at other heterochromatic loci, indicating that these factors are involved in a general silencing mechanism. Deletion of each gene enhanced silencing, implying that they negatively regulate silencing. To understand the molecular mechanism of Rhp6/Ups1 action in silencing, we are to investigate whether one of general silencing factors such as Swi6 and Rik1 is subject to ubiquitination by Rhp6/Ups1.

F118 Analysis of Physical Interaction between Two Recombinational Repair Proteins, Rhp51 and Rad22, in *Schizosaccharomyces pombe*

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In the budding yeast, RAD52 epistasis