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

group is involved in the homologous recombination pathway. Among the group, the Rad51 and Rad52 are regarded as key components for this pathway. The proteins are known to interact each other. However, the nature of this interaction and their role on recombinational repair are not fully understood. Here we investigated the role of interaction between Rhp51 and Rad22, a Rad51 and Rad52 homologues of *S. pombe*, respectively. Direct association of the two proteins was manifested both in vivo and in vitro, using the co-immunoprecipitation, GST-pull down and two-hybrid assays. Each protein also associates homotypically as well as heterotypically. In addition, the domains in each protein that mediate the both types of interactions were determined by two-hybrid analysis. To characterize the role of Rhp51/Rad22 interaction on recombinational repair, we isolated binding mutants of each Rhp51 and Rad22. Interestingly, we found that Rhp51/Rad22 interaction is crucial for the DNA repair ability of Rhp51, but Rhp51 self-interaction is not. These results suggest that direct binding of Rhp51 to Rad22 may be necessary for the proper DNA repair by Rhp51.

F119 Atf1 and Pcr1, Stress -Responsive Transcription Factors, Are Required for Heterochromatin Silencing from *Schizosaccharomyces pombe*

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Atf1 and Pcr1 contain bZIP DNA binding motive and belong to CREB/ATF family. They are required for expression of a variety of genes in sexual development and stresses and also for activation of M26 meiotic recombination hot spot. Here, we report that a null mutation of the *atf1*$^+$ reduces significantly silencing of *mat3-M* locus but conversely increase the transcriptional repression at centromere and telomeres. Likewise, *pcr1* reduces moderately silencing of *mat3-M* locus, implying that the silencing may require heterodimer formation between Atf1 and Pcr1. Deletion of atf1 and pcr1 increases the transition rates between repressed and derepressed states as revealed by colony color assay. This suggests that they are involved in establishment of silencing rather than in its maintenance. Chromatin immunoprecipitation (ChIP) assay revealed that both Atf1 and Pcr1 are associated in vivo with a flanking region of *mat3-M* containing their potential binding site. Thus, we suggest that Atf1 and Pcr1 involve in maximal silencing at mat locus by direct binding with their binding site and subsequently recruiting other silencing factors for formation of stable heterochromatin structure

F120 The Effect of PD 098059, Inhibitor of ERK2, in HeLa S3 Cells

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In the present study, we examined the effect of PD 098059, inhibitor of ERK(extracellular signal-regulated kinases) 2, in HeLa S_3 cells. The role of ERK2 considered to be important mediators of proliferative and apoptotic signals in serum-induced HeLa S_3 cell. Four assays were employed in this study : gel electrophoresis of isolated DNA, apoptotic cells, cell viability and western blot analysis. Apoptosis was detected by demonstration of DNA ladder pattern in agarose gel electrophoresis. Nuclear condensation and fragmentation, which are part of the early events of apoptosis, were evaluated by fluorescence microscopy. The cells were labeled with acridine orange/ethidium bromide. Viable cells were assessed by trypan blue dye exclusion method. Expression of ERK2 were