

Supplemental Methods

Strains, medium and cell cultivation

The *S. pombe* wild-type haploid strain *sp972* and all the mutant/**over-expressed** type strains were grown in YPD medium (2% glucose, 1% yeast extract, 2% peptone) at 30°C. The wild-type strain was used for construction of the cDNA library and transformations. ΔT , ΔH and $\Delta T-H$ strains were used to study the transcription of cluster I. *RRN7*, $\Delta DDB1$, $\Delta rqh1$, **Empty** and **OE-DDB1** strains were used to study the transcriptional co-regulations between box C/D snoRNA and RP genes. All the yeast strains were used for RNA and DNA analyses. *Escherichia coli* TG1 [*F'/supE hsdΔ5 thiΔ(lac-proAB)*] grown in 2YT liquid or solid medium (1.6% peptone, 1% yeast extract, 0.5% NaCl) was used for all cloning procedures.

Isolation of nuclei from *S. pombe* yeast

After centrifuging 2 L of culture and washing in S buffer containing 1.4 M sorbitol, 40 mM HEPES, 0.5 mM $MgCl_2$ and 1 mM PMSF, the cells were resuspended in 50 ml pretreatment buffer (S buffer containing 10 mM 2-mercaptoethanol) and incubated with gentle shaking at 30°C for 10 min. For 1 g wet cells, 20 mg lywallzyme was added and digested at 30°C for 3 h with gentle swirling. After digestion, spheroplasts were harvested by centrifugation at 3500 rpm for 5 min at 4°C and washed twice with ice-cold S buffer, then the spheroplast pellets were resuspended in 40 ml of ice-cold F buffer containing 18% Ficoll 400, 20 mM PIPES, 0.5 mM $MgCl_2$, 1 mM PMSF and lysed using a homogenizer. The supernatant of the spheroplast lysate was subsequently transferred to 50%, 40% and 30% Ficoll step gradients and centrifuged at 18000 rpm for 60 min at 2°C. Nuclei occur in the 40-50% interface.

Nuclear RNA extraction and construction of cDNA library

Total nuclear RNA was isolated and purified. A total of 8 μ g RNA was tailed with ATP using poly(A) polymerase. Reverse transcription was carried out in a 50 μ l reaction mixture containing 8 μ g RNA and 2 μ g oligo(dT) in the presence of 100 μ M dNTPs. After denaturation at 65°C for 5 min, the mixture was cooled to 42°C for 10 min, then 100 U of AMV reverse transcriptase (Promega) were added and incubated at 42°C for 40 min. The cDNA, synthesized by reverse transcription, was subsequently fractionated on a denaturing 8% polyacrylamide gel (8 M urea, 1 $\times$ TBE buffer). cDNA in the size range from ~60 to ~400 bp were excised from the gel, passively eluted and ethanol precipitated. Subsequently, 1 μ g of DNA was tailed with dGTP using 7 U of terminal deoxynucleotidyl transferase. cDNAs were

amplified using PCR with the forward primer poly(T), containing a HindIII restriction site, and the reverse primer poly(C)m containing BamHI restriction site. After digestion with HindIII and BamHI, the fragment was cloned into the plasmid pTZ18's corresponding restriction site, and the resulting plasmids were used to transform the strain *E. coli* TG1. After screening by filter hybridization, the sequences were determined.

Filter hybridization and isolation of clone

For exclusion of the 5.8S, 18S and 25S rRNA, oligonucleotides derived from these sequences were end-labeled with [γ -³²P]ATP and T4 polynucleotide kinase (Promega) and hybridized to DNA clones on filters. Hybridization was carried out in 5× SSC, 1% SDS, 5× Denhart's at 45°C for 12 hr. After washing twice at room temperature for 10 min in 15 mM sodium citrate buffer, pH 7.0, and 0.1% SDS, the filters were exposed to a phosphor screen and analyzed with a Typhoon 8600 variable mode imager.

5' RACE and 3' RACE assays

The transcription start site (TSS) and transcription terminating site (TTS) of the primary transcript of *S. pombe*'s seven snoRNA clusters were determined by 5' RACE and 3' RACE assays, using a 5' full RACE kit and a 3' full RACE kit, respectively (TaKaRa). For the 5' RACE assay, 2 µg total RNA treated with DNaseI was used in each reaction. Reverse transcription using random 9 primers was followed by nested PCR with common forward primers and specific reverse primers. The inner PCR products were detected by electrophoresis on a 1-2% agarose gel, then cloned and sequenced. For the 3' RACE assay, poly(A) + RNA was reverse transcribed with a 3' RACE adaptor primer. Nested PCR was performed with specific forward primers and common reverse primers using cDNA as the template. Then, the PCR products were electrophoresed, cloned and sequenced.