Characterization of the fission yeast ribosomal DNA binding factor: components share homology with Upstream Activating Factor and with SWI/SNF subunits

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ABSTRACT

A ribosomal DNA (rDNA) binding activity was previously characterized in fission yeast that recognized the upstream ribosomal RNA (rRNA) gene promoter in a sequence specific manner and which stimulated rRNA synthesis. It was found to share characteristics with Saccharomyces cerevisiae’s Upstream Activating Factor (UAF), an RNA polymerase I (pol I) transcriptional stimulatory factor. Putative fission yeast homologs of the S.cerevisiae UAF subunits, Rrn5p and Rrn10p, were identified. The Schizosaccharomyces pombe rDNA binding activity/transcriptional stimulatory activity was found to co-fractionate with both SpRrn5h and SpRrn10h. Analysis of polypeptides interacting with SpRrn10h uncovered a 27 kDa polypeptide (Spp27) homologous to a SWI/SNF component (now known to be homologous to Uaf30p). The contributions of the S.pombe and S.cerevisiae upstream rDNA promoter domains were assessed in cross-species transcriptional assays. Furthermore, comparative genomic analysis revealed putative Rrn5p, Rrn10p, Rrn9p and p27 homologs in multiple non-vertebrates. The S.pombe rDNA binding activity is proposed to be an RNA pol I specific SWI/SNF type factor.

INTRODUCTION

During active cellular growth, synthesis of the large ribosomal RNAs (rRNAs) accounts for nearly half of total RNA synthesis. This high level of rRNA synthesis is driven, in part, by the action of ribosomal DNA (rDNA) transcription factors that associate with the upstream region of rRNA gene promoters and/or intergenic regulatory sequences to mediate stimulation of this synthesis (1–3). In vertebrates, upstream binding factor (UBF), an HMG box protein, is such a factor that associates with rDNA promoters (4) and enhancers (5,6) in a sequence tolerant fashion (7) to increase levels of synthesis up to ~50-fold. UBF functions as a dimer (8); its activity is modified by phosphorylation (9,10), by acetylation (11,12) and by interaction with Retinoblastoma protein (13).

In baker’s yeast, an RNA polymerase I (pol I) transcriptional stimulatory factor, Upstream Activating Factor (UAF), was characterized both genetically and biochemically as being important for synthesis of the large rRNAs (14,15). It was found to stably associate with upstream rDNA promoter sequences in a sequence-specific manner and to consist of six subunits: Rrn5p, Rrn9p, Rrn10p, p30 (Uaf30p), and Histones H3 and H4 (14–16). Lesions in RRN5, RRN9 or RRN10 resulted in a severe decrease in growth rate and in synthesis of large rRNAs (15) and in an increase in switching of the catalytic enzyme for rRNA synthesis from RNA pol I to RNA polymerase II (pol II) (17).

It was unclear whether UBF was present in non-vertebrates or in lower eukaryotes and whether a UAF activity was present in higher eukaryotes. This study focuses on analysis of an rDNA binding factor in fission yeast that interacts specifically with the Schizosaccharomyces pombe upstream rDNA promoter and that increases levels of rRNA synthesis (18). Since it shares characteristics with Saccharomyces cerevisiae UAF, a search was conducted to identify potential S.pombe components of the rDNA binding factor based on homology to Rrn5p, Rrn9p and Rrn10p. Putative homologs were identified for Rrn5p and Rrn10p, and epitope-tagged versions were engineered for investigating molecular interactions and activities leading to species-specific activation of rRNA synthesis.

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MATERIALS AND METHODS

Identification of putative SpRrn5 and SpRrn10 homologs

Database searches of the S. pombe genome (Wellcome Trust Sanger Institute) uncovered putative homologs of S. cerevisiae Rrn5p and Rrn10p: SpRrn5h (S. pombe Rrn5p homolog, accession no. Q10360) and SpRrn10h (accession no. O14013, putative Rrn10p homolog). Searches for putative homologs of the S. pombe SpRrn5h and SpRrn10h (and later S. pombe p27) were also conducted in other available databases [those at NCBI; Neurospora crassa database at the Whitehead Institute; databases at DOE Joint Genome Institute including white rot fungus (Phanerochaete chrysosporium); Aspergillus fumigatus at TIGR; Candida albicans at the Stanford Genome Technology Center website, http://www-sequence.stanford.edu/group/candida; Cryptococcus neoformans at C. neoformans Genome Project, Stanford Genome Technology Center and The Institute for Genomic Research] and the Genolevures database (16) (http://cbi.labri.u-bordeaux.fr/Genolevures/advanced_blast.php3) using BLASTP, position-specific iterated BLAST (PSI-BLAST; 20) and TBLASTN. Putative homologs of SpRrn5h were identified in N. crassa [686 residues, contig 1.115, Neurospora Sequencing Project. Whitehead Institute/MIT Center for Genome Research (www-genome.wi.mit.edu; V: 3/12/01)], white rot fungus (504 residues, Scaffold 65, DOE Joint Genome Institute), A. fumigatus (primary sequence data was obtained from The Institute for Genomic Research website at http://www.tigr.org); C. neoformans (399 residues, CN11020013 AA, database: cne0_orsf011005), K. lactis (accession no. AL426913) and K. marxianus (accession no. AL426913). Putative SpRrn5h homologs include: C. albicans Rrn10h (Contig 6-1536), N. crassa (contig 2.273), A. fumigatus (contig 690), S. bayanus (AL402119), K. lactis (AL428395), K. marxianus (AL422400) and P. farinosa (AL417122). Multiple protein alignments were conducted using ClustalW (21) [http://searchlauncher.bcm.tmc.edu (Baylor College of Medicine)] and displayed using Boxshade (http://www.ch.embnet.org/).

Construction of pREP-Sp-MH-Rrn5h, pREP-Sp-FLAG-Rrn10h and pREP-Sp-MH-Rrn10h

The forward and reverse primers for PCR amplification of SpRrn5h were 5’-CCCATATGTCCTCTTTCAATAACCGG and 5’-GAGATCTCTCTTTAAGGGGATGAC, and the template was an S. pombe pACT cDNA library (22; purchased from American Type Culture Collection). For clonings in general, PCR products were first inserted into pCR2.1-TopoTM (Invitrogen) before subcloning into the target vector. The SpRrn5h PCR product was isolated as an NdeI/BgIII fragment and ligated into pREP42MH N (23) cleaved with NdeI and BamHI. Coding sequences of the putative S. pombe Rrn10 homolog were amplified from a S. pombe pACT cDNA library (since the coding sequences are interrupted by an intron) using the primers SpR10f9 GCCATAAGATCAAAATGCT and SpR10r10 Re2, CCGATCTGATCTGGTCTTTCTAAATCC. The PCR product was isolated as an NcoI-partial BamHI fragment and cloned into pREP-HA-FLAG cleaved with NcoI and BamHI. To construct pREP-HA-FLAG, coding sequences for the HA and the FLAG epitope tags were amplified from pGEM:HA-FLAG (kindly provided by Drs Alexander Hoffman and R. Roeder) with 5’-CGCGTCGACCATCATGGCCACAGATGCTC and 5’-GGAAATCCATGATCACCCCCCCC. This fragment was cleaved with NdeI and SalI, purified, and ligated to NdeI/SalI cleaved pREP, creating pREP-HA-FLAG. pREP-Sp-MH-Rrn10h was made by inserting a PCR fragment (primers SpR10f9 GCCATATGTCCTCTTTCAATAACCGG and SpR10r10 CGGATCTGATCTGGTCTTTCTAAATCC) into NdeI/BamHI cleaved pREP42MH N.

Preparation and fractionation of extracts from fission yeast

Whole cell extracts (WCEs) were prepared from -5 l of MP6-10B pREP-Sp-FLAG-Rrn10h pREP-Sp-MH-Rrn5h (‘Sp-MH-Rrn5h/Sp-FLAG-Rrn10h’) cells grown in selective Edinburgh minimal medium (24), as well as MP6-10B pREP-Sp-FLAG-p27 and pREP-Sp-MH-Rrn5h (‘Sp-MH-Rrn5h/Sp-FLAG-p27’/Sp-MH-Rrn5h) or pREP-Sp-MH-Rrn10h (‘Sp-MH-Rrn10h/Sp-MH-Rrn10h’). Control WCEs were prepared from 2.5 l of cells expressing just Sp-MH-Rrn10h, Sp-FLAG-Rrn10h or Sp-MH-Rrn5h. The extract proteins (94.1 mg, Sp-MH-Rrn5h/Sp-FLAG-Rrn10h; 89.1 mg, Sp-FLAG-p27/Sp-MH-Rrn5h; and 78 mg, Sp-FLAG-p27/Sp-MH-Rrn10h) were fractionated by ammonium sulfate precipitation, the pellet recovered and dialyzed, and proteins were then subjected to anion exchange chromatography using three 5 ml HiTrapQ™ columns (Amersham Pharmacia Biotech) as described (18). The columns were developed using a KCl step gradient [0.1 M, 0.35 M, 0.7 M KCl–1× column buffer (CB: 25 mM HEPES pH 7.9, 0.2 mM EDTA, 5 mM MgCl2, 20% glycerol, 1 mM PMSF and 1 mM DTT)]. All buffers contain PMSF and DTT, added just prior to use. The RNA pol I transcription activity eluted with 0.35 M KCl–1× CB. Peak fractions were pooled (27 mg total for ‘SpRrn5/SpRrn10h’; 31 mg for ‘SpRrn5/SpRrn10h’; and 24 mg for ‘SpRrn5/SpRrn10h’), converted to 1× wash buffer (WB: 25 mM HEPES pH 7.9, 0.15 M KCl and 20% glycerol) and rotated with 1 ml anti-FLAG™ M2 affinity matrix (Kodak/IBI) or 1 ml Ni-NTA agarose matrix (Ni-nitrilotriacetic acid; Qiagen; 1× WB, 0.1% NP-40) for 2 h at 4°C, followed by transfer to a column. For the M2 affinity matrix: following five, 1 ml washes with 1× WB, polyepitides were eluted with 1× WB–125 μg/ml FLAG peptide (five×1 ml). For the nickel affinity matrix, the column was washed five times with 1 ml WB, 0.1% NP-40, 10 mM imidazole, and myc-his-tagged proteins were eluted with 1× WB, 0.1% NP-40, 0.3 M imidazole. Approximately 30 mg of the control extracts (MP6-10B expressing just Sp-MH-Rrn5h; Sp-MH-Rrn10h or Sp-MH-Rrn10h) were fractionated as above and in Figure 2D [Sp-MH-Rrn10h (Fig. 2C)] or in Figure 7G [Sp-MH-Rrn5h (Fig. 7C) or Sp-MH-Rrn10h (Fig. 7F)] but using one 5 ml HiTrapQ™ matrix. Concentration and buffer exchange was performed by ultrafiltration using Centricon 10 units.

For western analysis, fractions were resolved on a 16 or 12% SDS–polyacrylamide gel and transferred to nitrocellulose membrane. Concentrations of fractions (in μg/μl) of Figure 2A and B (Sp-MH-Rrn5h/Sp-FLAG-Rrn10h) were Hi Trap Q (Q) – Q-0.1, 1.8 μg/μl; Q-0.35, 2.0; Q-0.7, 0.8; Ni-NTA FT, 1.3; Ni-NTA EL, 0.1; Figure 2C (Sp-FLAG-Rrn10h): Q-0.1, 1.9 μg/μl; Q-0.35, 1.8; Q-0.7, 0.6; Ni-NTA FT, 1.3;
Ni-NTA El, <0.1; Figure 7A and B (Sp-FLAG-p27/Sp-MHRrn5h): Q-0.1, 2.2 μg/μl; Q-0.35, 2.1; Q-0.7, 0.9; M2 aff.-FT, 1.5; M2 aff.-El, <0.1; Figure 7C (Sp-MHRrn5h): Q-0.1, 2.2 μg/μl; Q-0.35, 2.3; Q-0.7, 0.7; M2 aff.-FT, 1.6; M2 aff.-El, <0.1; Figure 7D and E (Sp-FLAG-p27/Sp-MHRrn10h), Q-0.1, 2.0 μg/μl; Q-0.35, 1.9; Q-0.7, 0.7; M2 aff.-FT, 1.5; M2 aff.-El, <0.1; Figure 7F (Sp-MHRrn10h): Q-0.1, 2.1 μg/μl; Q-0.35, 1.8; Q-0.7, 0.6; M2 aff.-FT, 1.6; M2 aff.-El, <0.1. Either anti-FLAG, M2 monoclonal antibody (Kodak/IBI; for Sp-FLAG-p27 and Sp-FLAG-p27) or anti-myc monoclonal antibody [9E10 (BabCo); Sp-MH-Rrn5h or Sp-MH-Rrn10h] was used at 1:1500 dilution for immunodetection as described (25), with the secondary peroxidase linked anti-mouse antibody at 1:5000 dilution. Antibody/polypeptide complexes were detected with Enhanced Chemiluminescent reagents (Amersham Pharmacia Biotech). Polypeptide size markers were Kaleidoscope and broad range standards (Bio-Rad).

Two-hybrid analysis

For cloning into the parental vectors pGAD and pGBDU (26), SpRrn5h sequences were amplified using 5′-GGAATTCCATGCTTTCTCAATAACCGGATATTAGT and 5′-TGAAGATCTTTTTAAGGGAGTGACAAC. The PCR fragment was isolated as an EcoRI/BglII fragment and inserted into pGBDU-C1, creating pGBD-SpRrn5h, while SpRrn10h cDNA was amplified using CGAATTCATGTCAAATCCAGACAGGCCTGGGTTTCCCAACTCGGC and CTGCTAGCTCTTTTCTTCAAAATTCTTCC. It was inserted into EcoRI/Sall cut pGBDU-C1 and pGAD-C1, creating pGBD-SpRrn5h and pGBD-SpRrn10h. Plasmids were transformed into PJ69-4A (MATa trpl-901 leu2-3,112 ura3-50 his3-200 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ (26), and activation of the three reporters, ADE2, HIS3 and lacZ, was assessed (M.Tripp and L.Pape, manuscript in preparation). One of interest contained coding sequences for a predicted 27 kDa polypeptide (Sanger Centre, SPCC285.17).

Construction of pREP-Sp-FLAG-p27 and full-length pGBDU-Spp27

The primers used to amplify full-length Sp.pombe p27 (Spp27) sequences from a Sp.pombe pACT cDNA library (to avoid amplifying the three introns) were 5′-CCCATGGCATGAGATCGAACACAGAC and 5′-CCGATCTCTCAAGCGGCGGTTGATCTCGCCGG. The resultant NcoI/BamHI fragment was cloned into pREP-HA-FLAG cut with the same enzymes. The primers 5′-CGGATCCATGGAGATCGAACACAGAC and 5′-CATGCTCTCAAGCGGCGGTTGATCTCGCCGG were used to amplify Spp27 cDNA for insertion into BamHI and BglII cleaved pGBDU-C1 as a BamHI/BglII fragment. The resultant plasmid was pGBDU-Spp27.

Alignment of Sp.pombe p27/S.cerevisiae Uaf30p homologs

Putative Sp.pombe p27 (AL031545) homologs include: white rot fungus (P.chrysosporium), DOE JGI, Scaffold 27; N.crassa p27 homolog, contig 1.821, NCU02204.1, Neurospora Sequencing Project, Whitehead Institute/MIT Center for Genome Research (www-genome.wi.mit.edu; V. 3/12/01); Drosophila melanogaster p27 homolog, AAF47684.1; Arabidopsis thaliana AAD43149.1 (386 amino acid); AAL24145 (385 amino acid) BAB01706 (452 amino acid), and ~13 additional ones; Bombyx mori (AU006413.1); C.servevisiae Uaf30p (YOR295W; 16), Caenorhabditis elegans p27 homolog (AA50726.1; Orzya sativa (AAK63939.1); Apis mellifera (bee; BI516000); Hordeum vulgare (BF621939); Chlamydomonas reinhardtii (BI528153); Chlamydia muridarum (NP_297118). Additional putative p27 homologs include cotton, tomato, corn, moss, soybean, C.albicans, A.fumigatus, Debaryomyces Hansenii, etc.

Electrophoretic mobility shift analysis with SpRrn5h/SpRrn10h fraction

A 32P-5′-end-labeled fragment (0.01 pmol) bearing the S.pombe rDNA promoter (~243 to +31; labeled at the BamHI and EcoRI sites in p3′Δ+31 and electrophoretically purified) was incubated with 7.5 μl of immunopurified Sp.pombe rDNA promoter sequences (±150, ±150 to +89; ±84, ±84 to +89; ±57, ±57 to +89 isolated from p5′Δ150, p5′Δ84, and p5′Δ57, or a 190 bp pBS SK+ HpalI fragment) at a 10- or 30-fold molar excess to the labeled rDNA promoter fragment. Electrophoresis was as described (18) except that the gel was 4% acrylamide–0.068% bisacrylamide–1× gel shift buffer–5% glycerol.

Construction of S.cerevisiae and Sp.pombe rDNA templates

The S.pombe wild-type template p3′Δ+31, containing a full promoter from ~243 to +31, was previously described (27,28). An analogous full-length S.cerevisiae rDNA template was constructed by PCR amplification of sequences between ~243 and +44 with ScF-243 5′-CCGGATCCCTTTCGCAATTTTTAAGATGAGTAT-GGCTTCTTCTTCTTTTCTTCCCTCCTTTCTCTCTCTTCGGA-3′ and Scr+44 5′-CCGGAAATTAAGATGAGTAT-GGCTTCTTCTTCTTTTCTTCCCTCCTTTCTCTCTCTTCGGA-3′. Template DNA was YEPrR8 (kindly sent by Dr J. Warner; 29). The fragment was cleaved with BamHI and EcoRI, gel isolated, and ligated between the BamHI and EcoRI sites of pBS SK+ to yield Sc-C243+/44. PCR amplifications were cycled at: 94°C, 1 min; 55°C, 2 min; 72°C, 2 min for 27 cycles. A BamHI–PstI fragment containing sequences from ~243 to ~56 of the S.cerevisiae rDNA promoter was amplified with ScF-243–Scr-56, 5′-GAAGATCGACCCCTTATCAACAAATTTAAA-ATC. Schizosaccharomyces pombe rDNA sequences from ~99 to +31 were amplified with SpF-49 5′-GAAGATCGACCCCTTATCAACAAATTTAAA-ATC. The PCR product was cleaved with PstI, isolated and ligated into PstI cleaved pSc-C243–Scr-56. The orientation of the S.pombe rDNA was assessed by dyeoxy sequence analysis. The primers ScR+44 (see above) and ScF-50, 5′-GAAGATCGACCCCTTATCAACAAATTTAAA-ATC were used to amplify the S.cerevisiae core rDNA promoter (~50 to +44); the fragment was cleaved with PstI and EcoRI and inserted into pBS SK+ linearized with these same enzymes, to create ‘Sc core’.
The *S. pombe* upstream promoter region from –243 to –56 was amplified with T3 and SpR-56 (5′-AGAAGTACGGAG-TTTCCGATCCATCCATCGG) primers, using p3Δ+31 as template. Trimolecular ligation of the BamHI–PstI fragment Sp-243/-56, the PstI–EcoRI fragment Sc-50/+44 and vector pBS SK+ cleaved with BamHI and EcoRI yielded the hybrid template Sp/Sc. The core promoter region of *S. pombe*, from –57 to +89, is in p5Δ-57 (28). Plasmid DNAs were purified and concentrations quantitated as described (18). To construct LS-106/-97, 3′-107, 5′-CCGATCCGGTCTTTCTTCAACAA-AAG-3′ was used with T3 to amplify rDNA from 3′Δ+31. The fragment was isolated as a BamHI/XhoI fragment and inserted into BamHI/XhoI cleaved 5′Δ-97 (sequences from –106 to –97 were AACGATCCG instead of the wild-type GGAGGAGA-TAT). For LS-68/-58, 5′-CCGATCCGGTCTCTTCAACAA-ATTTCGTCATTTCGG) primers, using p3Δ-57 at the unique BamHI site (position +76 on the template strand), cleavage with calf-alkaline phosphatase, 5′ (27). The probe for detecting specific transcripts from p3Δ-57 and isolation of the single-stranded fragment as described (27). Plasmid DNAs were puriﬁed based on association of activity with a tagged SpRpa43 homolog and shown to contain a fraction with transcription initiation factor activity [afﬁnity puriﬁcation of Sp-FLAG-Rrn10h and associated polypeptides.](https://academic.oup.com/nar/article/30/24/5347/1077713)

**RESULTS**

Synthesis of wild-type levels of large rRNAs in fission yeast is dependent on the presence of the upstream rDNA promoter domain (28) and on a sequence-speciﬁc rDNA binding activity that stimulates rDNA transcriptional initiation (18). Since several properties of the fission yeast rDNA binding activity were reminiscent of *S. cerevisiae* UAF (14,15), *S. pombe* polypeptides uncovered as potential homologs of *S. cerevisiae* UAF subunits Rm5p and Rrn10p were molecularly analyzed.

Characterization and molecular tagging of SpRrn5h and SpRrn10h

Alignment of a putative fission yeast Rm5p homolog with *S. cerevisiae* Rm5p is shown in Figure 1A. The putative *S. pombe* Rm5p homolog (SpRrn5h, 556 residues) has a calculated molecular weight of 64.0 kDa and a 147 amino acid C-terminus not present in *S. cerevisiae* Rm5p. The *S. cerevisiae* Rm5p and putative *S. pombe* homolog share 28% identical and 41% similar residues over 176 amino acids (E-value, 6 × 10⁻⁵ in a PSI-BLAST search of fungal sequences with *S. cerevisiae* Rm5p as query). In addition, SpRrn5h was shown to localize to the nucleolus (31). SpRrn5h contains a domain of particular interest: a SANT domain, located between residues 71 and 112 (E-value, 1 × 10⁻⁴, RPS-BLAST). The SANT domain was originally identiﬁed from SW13, ADA2, N-CoR and TFIIB*, and it overlaps a myb domain (32).

When fungal protein coding sequences are queried with *S. cerevisiae* Rm10p, a putative *S. pombe* homolog (SpRrn10h) is aligned, yielding an E-value of 1.4 [PSI-BLAST (20)]. Although this value is less than threshold, the putative SpRrn10h is the top match besides Rrn10p itself (see Fig. 1B). The putative SpRrn10h shares 29% identical and 49% similar residues with *S. cerevisiae* Rrn10p over 55 residues. Furthermore, when fungal sequences are searched with SpRrn10h as the query, *S. cerevisiae* Rrn10p is the top match after SpRrn10h (E-value, 0.61, PSI-BLAST).

Sp-FLAG-Rrn10h cofractionates with Sp-MH-Rrn5h

A fission yeast strain, MP6-10B pREP-Sp-MH-Rrn5h pREP-Sp-FLAG-Rrn10h, was engineered to express N-terminal myc-epitope and poly-histidine (MH)-tagged SpRrn5h fusion protein and FLAG-tagged SpRrn10h. Fractionation of WCE prepared from these cells was conducted to determine whether the putative SpRrn5h and SpRrn10h copuriﬁed with each other, with transcriptional stimulatory activity, and/or with rDNA binding activity (Fig. 2D). Western analysis of WCE proteins revealed an anti-myc antibody immunoreactive polypeptide with an apparent molecular weight of ~75 kDa (Fig. 2A, lane 1), corresponding to Sp-MH-Rrn5h (its calculated molecular weight is 68.3 kDa), while a 15.1 kDa polypeptide, corresponding to Sp-MH-Rrn5h (its calculated molecular weight is 68.3 kDa), was detected with M2 (anti-FLAG) monoclonal antibodies (Fig. 2B, lane 1). Neither of these immunoreactive polypeptides was detected in WCE prepared from control *S. pombe* cells (data not shown).

WCE proteins were subjected to ammonium sulfate precipitation, followed by fractionation on the anion exchange matrix, HiTrapQ™, and the nickel afﬁnity matrix as described (18). Polypeptides that eluted from the HiTrapQ matrices with 0.35 M KCl column buffer contained all factors required for specific rRNA synthesis (18,25), as well as the putative SpRrn5h and SpRrn10h (as seen in the immunoblot of Fig. 2A and B, lanes 3). The peak fractions of the 0.35 M KCl HiTrapQ eluate were pooled, applied to a nickel afﬁnity matrix and, following extensive washing of the matrix, polypeptides were eluted with 0.3 M imidazole buffer. In a parallel fractionation, extracts were prepared and fractionated as above, but the afﬁnity puriﬁcation in this alternate fractionation was an M2 (anti-FLAG) afﬁnity matrix for puriﬁcation of Sp-FLAG-Rrn10h and associated polypeptides.
As seen in Figure 2A, Sp-MH-Rrn5h (‘SpRrn5h’) was detected in fractions eluted from nickel affinity matrix with 0.3 M imidazole, as expected (lane 6, ‘Ni-NTA El’; compare to lane 5, ‘Ni-NTA FT’). A parallel immunoblot of proteins in the nickel affinity purified fractions was challenged with anti-FLAG (M2) antibody and revealed Sp-FLAG-Rrn10h largely in the bound fraction (‘Ni-NTA El’, Fig. 2B, lane 6) with a portion in the flow-through fraction (‘Ni-NTA FT’, lane 5). Further evidence for interaction came from co-immunoprecipitation analysis of Sp-MH-Rrn5h with Sp-FLAG-Rrn10h (data not shown). To assess the extent of binding of the putative Sp-FLAG-Rrn10h to the Ni-NTA matrix in the absence of Sp-MH-Rrn5h, WCE was prepared from S.pombe expressing just Sp-FLAG-Rrn10h and fractionated as in Figure 2D. As seen in Figure 2C, lane 5, SpRrn10h does not bind the Ni-NTA matrix in the absence of Sp-MH-Rrn5h but is present in the flow-through fraction (compare lane 5 with 6).

Affinity purified SpRrn5h/SpRrn10h fraction harbors sequence-specific rDNA binding and transcriptional stimulatory activities

Subsequently, the affinity purified Sp-MH-Rrn5h/Sp-FLAG-Rrn10h fraction (‘SpR5/SpR10’) was tested for its ability to confer stimulation of transcription supported by a full-length S.pombe rRNA gene promoter in in vitro reactions. These reactions were conducted under stringent conditions that reflect requirements for the upstream rDNA promoter region and for stimulatory transcription factors in directing efficient initiation. Correctly initiated rRNAs are represented by 76 bp S1-resistant products. Virtually no accurately initiated rDNA transcripts are detected when reactions contain either the RNA pol I fraction (RNA pol I/ S.pombe Rrn3p; see Fig. 3A, lane 2) or RNA pol I and the rDNA transcription initiation factor [‘init. factor’, lane 3 (28)]. However, when the affinity purified SpRrn5h/SpRrn10h fraction was also present in the reactions, the level of rDNA transcriptional initiation was significantly stimulated (see lane 4). The SpRrn5h/SpRrn10h fraction does not support correct initiation on its own (data not shown). Furthermore, Sp-MH-Rrn5h as well as the rDNA transcriptional stimulatory activity were found to immunopurify with Sp-FLAG-Rrn10h using an anti-FLAG affinity matrix (data not shown).

If SpRrn10h and SpRrn5h are components of the S.pombe sequence-specific rDNA binding activity, this activity should copurify with these tagged polypeptides. The presence of an rDNA binding activity was assessed by electrophoretic mobility shift analysis. When an end-labeled fragment bearing S.cerevisiae rDNA promoter sequences from ±243 to +31 was incubated in the presence of the affinity purified SpRrn5h/SpRrn10h fraction, the level of rDNA transcriptional initiation was significantly stimulated (see lane 4). The SpRrn5h/SpRrn10h fraction does not support correct initiation on its own (data not shown). Furthermore, Sp-MH-Rrn5h as well as the rDNA transcriptional stimulatory activity were found to immunopurify with Sp-FLAG-Rrn10h using an anti-FLAG affinity matrix (data not shown).

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Lane 1, ~120 was performed with anti-myc (A) or anti-FLAG (B) monoclonal antibodies. Acrylamide gel, transferred to nitrocellulose membrane, and western analysis.

3.0 M KCl ± 1

Thus, the 5′ complex (while 30-fold molar excess of ±57 to +89 does; lane 35) does not affect formation of the complex (while 30-fold molar excess of ±57 to +89 does; lane 35). In contrast, LS-35/-28, bearing non-specific bases between ~35 and ~28 (28), is only somewhat impaired (compare lane 3 with its wild-type control, lane 4).

Analysis of cross-species function of *S. cerevisiae* and *S. pombe* upstream rDNA promoter regions

Since the respective *S. cerevisiae* or *S. pombe* upstream rDNA promoter domains mediate the effects of *S. cerevisiae* UAF (15) or the *S. pombe* rDNA binding activity (18), the cross-species activities of these domains were tested in *in vitro* *S. cerevisiae* and *S. pombe* transcription assays on templates bearing hybrid *S. pombe*/*S. cerevisiae* rDNA promoters. The hybrid templates bore the *S. pombe* upstream rDNA promoter fused to an *S. cerevisiae* core rDNA promoter (Sp/Sc) or the *S. cerevisiae* upstream promoter fused to the *S. pombe* core promoter (Sc/Sp), while the control rDNA templates consisted of full-length *S. pombe* (Sp) or *S. cerevisiae* rDNA promoters (Sc) or the ‘Sc core’ and ‘Sp core’ rDNA templates that lacked the upstream promoter domains entirely (see Fig. 4D and E).

When the promoter strength of this series was assessed in *S. pombe* in vitro transcription reactions (see Fig. 4B), no correctly initiated rRNAs were detected supported by the *S. cerevisiae* core (Sc core; lanes 3 and 4) or full-length rDNA promoter (Sc; lanes 1 and 2). In contrast, the Sp/Sc template does direct accurate rRNA synthesis (see Fig. 4B, lanes 5 and 6), and its start site was fine-mapped to +1 (data not shown). This demonstrates that the *S. pombe* upstream rDNA promoter can mediate correct initiation of the *S. cerevisiae* core rDNA promoter with *S. pombe* RNA pol I transcription factors. In contrast, a cis-located *S. cerevisiae* upstream rDNA promoter (Sc/Sp) functions to repress basal level transcription of the *S. pombe* core rDNA promoter (Sp core) (see Fig. 4B, lanes 7 and 8, compare to Sp, lane 10, and Fig. 4C, compare lane 4 with lanes 1 and 2). The molecular basis underlying this repressive effect is unknown.

When transcription reactions were conducted with *S. cerevisiae* WCE components, the *S. cerevisiae* full-length rDNA promoter (‘Sc’), as expected, supported efficient transcriptional initiation (see Fig. 5, lanes 3 and 4), while levels supported by the *S. cerevisiae* core rDNA promoter (from ~49 to +44) were reduced 6-fold or more (compare lane 2 with lane 4, and lane 1 with lane 3, Figure 5). A cis-located *S. pombe* upstream rDNA promoter mediated a modest increase in transcription supported by the *S. cerevisiae* rDNA core promoter [compare lanes 1 and 2 (Sc core) to lanes 5 and 6 (Sp/Sc)]. In the Sc/Sp template, the cis-located *S. cerevisiae* upstream rDNA promoter domain increases transcription supported by the *S. pombe* rDNA promoter 50% compared to the wild-type Sp template (compare lane 9 with lane 11) and several-fold more compared to Sp core (lanes 12 and 13). As seen previously, transcription of the *S. pombe* rDNA promoter initiates at an altered site [see Fig. 5, lanes 8 to 13 (25)] in
S. cerevisiae in vitro transcription reactions. A minor transcript supported by Sc/Sp (lane 9) may represent initiation at the correct +1 site or may be due to non-specific initiation.

A 27 kDa S. pombe polypeptide interacts in vivo with SpRrn10h

To investigate interactions of the putative SpRrn10h and SpRrn5h as well as to search for specifically interacting polypeptides, their coding sequences were cloned in frame with the GAL4 DNA binding domain or activation domain in the vectors pGBDU and pGAD (26). In a large-scale two-hybrid screen, polypeptides that specifically associated with SpRrn10h were searched for in a library of S. pombe GAL4AD-cDNAs (Clontech). One of the GAL4AD-cDNAs that encoded a fusion protein capable of activating three independent reporters together with GAL4BD-SpRrn10h, indicating interaction, was found to encode a ~27 kDa polypeptide. This S. pombe 27 kDa polypeptide was homologous to S. cerevisiae YOR295W [now reported as a subunit of UAF, Uaf30p (16)] and YMR233W (16) and shared some homology with the mammalian ~60 kDa SWI/SNF subunit (33). As seen in Figure 6, the activation of the ADE2 and the HIS3 reporters in PJ69-4A bearing both pGBDU-SpRrn10h (SpRrn10h) and pGAD-Spp27 (Spp27) allowed growth on medium lacking adenine or histidine (the lacZ reporter was also activated; data not shown). In contrast, no activation was observed in strains expressing GAL4AD-Spp27 (Spp27) and GAL4BD-SpRrn5h (SpRrn5h); GAL4BD-Spp27 and S. cerevisiae GAL4AD-ScRrn9p (ScRrn9p); Spp27 and vector; GAL4DB-SpRrn10h (SpRrn10h) and ScRrn9p; or SpRrn10h and SpRrn5h. The reason for the negative two-hybrid result between SpRrn5h and SpRrn10 is unknown, since they co-fractionate with each other (perhaps mediated by another associated factor) and with the S. pombe rDNA binding activity. Furthermore, the documented interaction of S. cerevisiae Rrn9p with Rrn10p (34,35) appears to be species-specific in nature and is not seen between S. cerevisiae Rrn9p and S. pombe SpRrn10h.

Sp-FLAG-p27 cofractionates with Sp-MH-Rrn5h and with Sp-MH-Rrn10h

To test whether Spp27 co-fractionated with SpRrn5h—as expected if Spp27 were a component of the rDNA binding complex—pREP-Sp-FLAG-p27 encoding N-terminal FLAG-tagged Spp27 was introduced into S. pombe MP6-10B together with pREP-Sp-MH-Rrn5h. WCE was prepared and fractionated as described above, except that the affinity purification step used anti-FLAG affinity matrix (see Fig. 7G). Duplicate aliquots of the fractions were resolved on 16 and 12% SDS–polyacrylamide gels, transferred to nitrocellulose membrane and challenged with anti-FLAG (Fig. 7A) or anti-myc (Fig. 7B) monoclonal antibodies. Both Sp-MH-Rrn5h and Sp-FLAG-p27 co-eluted from the HiTrap Q matrix (Fig. 7A and B, lane 3) together with the RNA pol I transcriptional activity.
S. pombe rDNA core promoter (Sc core); lanes 5 and 6, hybrid template with S. cerevisiae wild-type (m). Transcription reactions were conducted with S. pombe core (Sc/Sp); and lane 10, wild-type S. pombe (Sp/Sc); lanes 7 and 8, S. cerevisiae the reaction (0.5 or 1.25 m). S. pombe supported by Sc/Sp (lanes 1 and 2), full-length C single-stranded probes had a comparable specific activity. (Pst rDNA sequences are underlined, Schizosaccharomyces pombe E rDNA templates used in this study. (correctly initiated rRNAs from Sp core template. (lanes 1±3, while `+1 core' is a 140 nt S1-protected fragment representing correctly initiated rRNAs from templates in LS-35/-28 is 76 nt (`+1*'), and is 140 nt for LS-106/-97 and LS-68/-57 (`+31'). Template concentration was 125 ng/ml. (Figure 4. In vitro transcriptional analysis in S. pombe WCE supported by LS-106/-97 (lane 1), LS-68/-58 (lane 2), LS-35/-28 (lane 3 (28)) and the wild-type 3′A+31 (lane 4) was assessed by S1 analysis. The S1-protected fragment representing correctly initiated rRNAs supported by 3′A+31 and LS-35/-28 is 76 nt (+1*), and is 140 nt for LS-106/-97 and LS-68/-57 (+31). Template concentration was 125 ng/ml. (B) Analysis of transcriptional strength of hybrid S.cerevisiae–S.pombe rDNA promoters. Transcription reactions were conducted with S. pombe S-100 extract and templates at 20 or 50 ng per reaction (0.5 or 1.25 μg/ml). Lanes 1 and 2, wild-type S. cerevisiae rDNA template (Sc); lanes 3 and 4, S. cerevisiae rDNA core promoter (Sc core); lanes 5 and 6, hybrid template with S. pombe upstream rDNA promoter and S. cerevisiae rDNA core promoter (Sp/Sc); lanes 7 and 8, S. cerevisiae upstream rDNA promoter and S. pombe core (Sc/Sp); and lane 10, wild-type S. pombe rDNA template (Sp). Both single-stranded probes had a comparable specific activity. (C) Transcription supported by Sc/Sp (lanes 1 and 2), full-length S. pombe rDNA promoter (lane 3), and Sp core (lane 4). The 20 or 50 refers to ng template present in the reaction (0.5 or 1.25 μg/ml template). The +1* marks the 76 nt S1 protected fragment representing correctly initiated rRNAs from templates in lanes 1–3, while `+1 core' is a 140 nt S1-protected fragment representing correctly initiated rRNAs from Sp core template. (D) Diagram of the hybrid rDNA templates used in this study. (E) Sequence of the hybrid and wild-type rDNA promoters at the junction site. Schizosaccharomyces pombe rDNA sequences are underlined, PstI linker bases are in italics and S. cerevisiae rDNA sequences are plain. (18). Peak fractions eluted with 0.35 M KCl–1× CB were applied to an anti-FLAG, M2 monoclonal antibody affinity matrix. Following extensive washing, bound polypeptides were eluted with FLAG peptide. As expected, Sp-FLAG-p27 is specifically bound to the M2 affinity matrix and dissociates upon addition of FLAG peptide (Fig. 7A, lane 6). The reason for the discrepancy between its apparent molecular weight of ~42 kDa and the calculated value of 30.1 kDa is unknown, as is the presumptive modification underlying the slower migrating immunoreactive band of ~44 kDa. Sp-MH-Rrn5h (~75 kDa apparent molecular weight) is detected in the fraction bound to the M2 affinity matrix, presumably via interactions with Sp-FLAG-p27 ['SpRrn5h', Fig. 7B, lane 6, ‘M2 aff.-El' for Sp-FLAG-p27, see Fig. 7A, lane 6 (‘Spp27')], although a portion is also present in the flowthrough fraction (‘M2 aff.-FT', Fig. 7B, lane 5). In addition, fractionation of an extract prepared from fission yeast expressing just the epitope-tagged Sp-MH-Rrn5h revealed that it does not specifically bind to the M2 affinity matrix in the absence of Sp-FLAG-p27 (Fig. 7C). Thus, Sp-MH-Rrn5h co-fractionates with Sp-FLAG-p27, suggesting that SpRrn5h, SpRnm10h and S. pombe p27 (SpSpp27) associate in a UAF-like complex.

WCEs were also prepared and fractionated from S. pombe expressing Sp-FLAG-p27 and Sp-MH-Rnm10h (see Fig. 7D and E, lane 1), as above. Sp-MH-Rnm10h co-elutes from the HiTrap Q matrix with Sp-FLAG-p27 and is largely present in the bound fraction of the M2 affinity matrix (see Fig. 7D and E, lanes 3 and 6), consistent with their in vivo interaction (Fig. 6). In contrast, when WCE prepared from S. pombe cells expressing just Sp-MH-Rnm10h was fractionated as in Figure 7G, Sp-MH-Rnm10h was found in the flowthrough fraction of the M2 affinity matrix (Fig. 7F, lane 5).

The SpSpp27/SpSpp5h fraction was assayed to determine whether it contained an activity leading to stimulated levels of rDNA transcriptional initiation. Addition of the affinity purified SpSpp27/SpSpp5h fraction to a reaction containing basal level pol I transcription initiation components and a full-length rDNA promoter resulted in a significant increase in rRNA synthesis (compare lane 5 with lane 3, Fig. 3A), while it supported no initiation itself (data not shown). This SpSpp27/SpSpp5h fraction also harbored sequence specific rDNA binding activity (data not shown).

Identification of p27, SpRrn5h and SpRnm10h homologs in other eukaryotes

Sequence alignment of the S. pombe Spp27 with other eukaryotic homologs is seen in Figure 8A (and Supplementary Material, Fig. 9A). Extensive database searches reveal that non-vertebrate cells have a clearly identifiable homolog of Spp27 as well as a homolog of the SWI/SNF 60 kDa subunit, the latter polypeptide, however, shares less sequence homology with Spp27 than the putative SpSpp27 homologs. A clearly identifiable homolog of SpSpp27 was not found in the proteomes of vertebrates, although the 60 kDa SWI/SNF subunit homolog was.

The genomes of N. crassa, P. chrysosporium (white rot fungus), A. fumigatus, C. neoformans, K. marxianus and K. lactis encode putative Rrn5p homologs that align with S. pombe SpRrh5h and S. cerevisiae Rrm5p as seen in Figure 8B. (Full-length alignments are shown in Supplementary Material,
Fig. 5. Transcriptional analysis of hybrid rDNA promoters conducted in *S. cerevisiae* S-100 extracts. The rDNA templates bearing Sc core (lanes 1 and 2), Sc (lanes 3 and 4), Sp/Sc (lanes 5 and 6), Sc/Sp (lanes 8 and 9), Sp (lanes 10 and 11) and Sc core (lanes 12 and 13) rDNA promoters were transcribed *in vitro* in an *S. cerevisiae* transcription extract, with the final template concentration at 0.5 µg/ml (20 ng; lanes 1, 3, 5, 8, 10 and 12) or 1.25 µg/ml (50 ng; lanes 2, 4, 6, 9, 11 and 13). Correctly initiated *S. cerevisiae* rRNAs are represented by a 76 nt S1 protected fragment (lanes 1–6). Lanes 8–11, the 67 nt S1 protected fragment represents initiation from Sc/Sp and Sp at +10 (fine-mapped; data not shown). Lanes 12 and 13: The 130 nt S1-protected fragment represents rRNAs initiated at +10 from Sc core. Transcriptional efficiency is noted relative to Sc 50 (lane 4) for lanes 1–6, and relative to Sp 50 (lane 11) for lanes 8–11.

Fig. 6. Two-hybrid analysis of interactions with SpRrn10h. Transformants expressing GAL4 DNA binding domain (GBD)-SpRrn10h and GAL4 activation domain (GAD)-SpRrn5h, Spp27 or ScRrn9p fusion proteins. The top panels show growth of transformants on medium lacking adenine, leucine and uracil (left) or lacking histidine, leucine and uracil, with 5 mM 3 amino triazole (right). Transformants are PJ69-4A pGBDU-SpRrn10h pGADSpp27 (SpRrn10h × Spp27), PJ69-4A pGBDU-Spp27 × pGADSpRrn5h (Spp27 × SpRrn5h), PJ69-4A pGBDU-Spp27 pGADScRrn9p (Spp27 × ScRrn9p), PJ69-4A pGBDU-Spp27 × pGAD-C1 (SpRrn10h × vector), PJ69-4A pGBDU-SpRrn10h pGADScRrn9p (SpRrn10h × ScRrn9p), and PJ69-4A pGBDU-SpRrn10h pGADspRrn9p (SpRrn10h × SpRrn5h). A diagram in the bottom panel depicts positions of the transformants: the top polypeptide listed of each pair is expressed as a fusion protein with the GAL4 DNA binding domain; the bottom of each pair as a fusion with the GAL4 activation domain.

DISCUSSION

Stimulation of rRNA synthesis in eukaryotes is mediated by cis-acting sequences in the upstream rDNA promoter as well as by the stimulatory factors, UBF in vertebrates and UAF (15) in *S. cerevisiae* (1–3 and references therein). These factors are vastly different in subunit structure: UBF is a homodimer composed of UBF1 or UBF2 molecules, while UAF is composed of six subunits: Rrn5p, Rrn9p, Rrn10p (15), histones H3 and H4 (14) and Uaf30p (16). UBF is abundant (up to 100 000 molecules per cell), while UAF is present at ~200 copies per cell (15). Both UAF and the *S. pombe* rDNA binding activity recognize their species’ upstream rDNA promoter in a sequence-specific manner (15,18), in contrast to UBF, which associates with rDNA in a sequence tolerant fashion (7,36). Both serve important roles in stimulating levels rRNA synthesis.
The presence of a UAF-like activity and UAF subunit homologs that co-purify with this activity in *S. pombe* demonstrate that UAF is not restricted to *S. cerevisiae*. As reported here, epitope-tagged versions of putative fission yeast SpRrn5h, SpRrn10h and Spp27 were found to co-fractionate with each other and with the *S. pombe* rDNA binding/rDNA transcriptional stimulatory activity. A fundamental question raised when UAF was first characterized (15) was whether other eukaryotes harbor UAF, and if they do, whether they regulate levels of rRNA synthesis via both UBF and UAF? While clear homologs of Uaf30p/S. pombe p27 have been found in invertebrates, including fungi, plants, *D. melanogaster*, silk worm, etc., putative Rrn5p homologs and Rrn10p homologs have only been identified in *S. pombe* and other fungi. These include representatives of basidiomycetes (white rot fungus) and ascomycetes: archaeascomycetes (*S. pombe*), hemiascomycetes (*S. cerevisiae*, *K. lactis*, *C. albicans*, etc.), and euascomycetes (*N. crassa*). The apparent lack of non-fungal, invertebrate homologs of Rrn5p and Rrn10p may be due to considerable sequence divergence at the primary structure level. If so, purification of UAF-like complexes via a tagged Spp27 or UAF30p homologous subunit could facilitate determination of the presence of such complexes as well as their subunit composition. Since disruption of YMR233W did not affect cell growth or rRNA transcription despite its shared homology with UAF30p (16), the Spp27 homologs have been called p27h until it is demonstrated that they play a role in rRNA synthesis. *Chlamydia*, a bacterial human pathogen, has the SWIB-Toxoisomerase I fusion (16) and a ‘stand-alone SWIB’ protein (37) that are Spp27 homologs among other chromatin-associated genes phylogenetically derived from eukaryotes.

It is of interest that at least two of the components of the *S. pombe* rDNA binding factor share homology with components of the SWI/SNF complex. SpRrn5h bears a SANT domain [first noticed in the SWI/SNF subunit SNF5 of *D. melanogaster*, and a TFIIIB component (32)]. Homology to Swi3p, however, extends beyond this domain (see Supplementary Material, Fig. 10). One function of a SANT domain was recently reported: a SANT domain in SMRT and N-CoR was shown to be important for interaction with and activation of histone deacetylase (38).

A second component of the *S. pombe* rDNA binding activity also bears homology to a SWI/SNF subunit: the Rrp10h interacting factor SpSpp27 to SWI/SNF 60 kDa (16; this paper). Taken together, these observations suggest that the *S. pombe* rDNA binding activity that co-fractionates with tagged SpRrn10h, SpRrn5h, and Spp27 (a UAF30p homolog) functions in chromatin remodeling and is targeted to the upstream rDNA promoter by its sequence-specific recognition of this region. If so, its ability to stimulate rDNA transcription may result, at least in part, from such chromatin remodeling activity (reviewed in 39–41).

While a homolog of the fourth RNA pol I specific subunit of UAF, Rrn9p, has yet to be characterized in *S. pombe*, putative Rrn9p homologs have been identified in multiple yeasts (see Supplementary Material, Fig. 11A and B). An intriguing observation obtained in a search of the *S. pombe* proteome with the putative *Kluyveromyces thermotolerans* Rrn9p homolog, KtRrn9h, as query was that *S. pombe* Snf2p was the top match.
(Supplementary Material, Fig. 11C); the putative A. fumigatus Rrn9h was shown to align with Snf2 as well (see Supplementary Material, Fig. 11D). However, since the homologies seen were limited, it remains to be tested whether fungal Rrn9p share any activity of Snf2p.

The polypeptides with the highest degree of homology to Spp27 are clearly distinct from the mammalian 60 kDa SWI/SNF subunit class. While Spp27 shares some homology with this SWI/SNF subunit, non-vertebrates including S. pombe, S. cerevisiae, D. melanogaster, C. elegans and A. thaliana, have Spp27 homologous coding sequences (see Fig. 8A). A search of eukaryotic coding sequences with these Spp27 homologs reveals that they align, in general, to yield E-values significantly higher with each other than with the SWI/SNF 60 kDa subunit homologs.

**Figure 8.** (A) Multiple alignment of S. pombe p27 (Sp.p27; 117–190 of 233 residues total) with putative homologs: white rot fungus (wrf.p27h; 264–337 of 363 total), N. crassa (Nc.p27h 186–259 of 265 total), A. thaliana (At.p27h; 251–324 of 386; note—there are 16 putative A. thaliana p27 homologs), O. sativa (Os.p27h; 67–141 of 144 total), H. vulgare (Hv.p27h), C. maralbum (Cm.p27h; 9–83 of 86 total), S. cerevisiae Uaf30P (Sc.UAF30, 120–192 of 228 total), D. melanogaster (Dm.p27h; 168–241 of 244 total), bee (bee.p27h), B. mori (Bm.p27h), C. elegans (Ce.p27h; 264–337 of 415 total) and C. reinhardtii (Cr.p27h). (B) Multiple alignment of S. pombe SpRrn5h (residues 68–160) and S. cerevisiae ScRrn5p (56–139) with putative Rrn5p homologs: K. marxianus KmRrn5h, K. lactis KlRrn5h, white rot fungus wrfRrn5h (66–165), C. neoformans CnRrn5h (52–147), A. fumigatus AfRrn5h (108–196) and N. crassa NcRrn5h (182–275). SpRrn5h, NcRrn5h, wrfRrn5h and AfRrn5h have clearly identifiable SANT domains: SpRrn5h, between residues 71 and 112 (E-value \(1 \times 10^{-4}\) RPS-BLAST (20)); N. crassa Rrn5h, between 147 and 185 (E-value \(1.3 \times 10^{-2}\)); white rot fungus Rrn5h, between 72 and 107 (E-value \(6.4 \times 10^{-2}\)) and A. fumigatus from 114 to 158 (E-value \(2.1 \times 10^{-2}\)). All have conserved residues in this domain. (C) Alignment of putative Rrn10p homologs: S. cerevisiae ScRrn10p (ScRrn10p, 93–143 of 183 total residues), K. marxianus KmRrn10h, K. lactis KlRrn10h (49–96 of 125), P. farinosa PfRrn10h (93–143 of 183 total), C. albicans CaRrn10h (residues 102–155 of 195 total), A. fumigatus AfRrn10h, N. crassa NcRrn10h (residues 89–157, with 7 residues removed ‘//’ (of 261 total)), and S. pombe SpRrn10h (residues 39–87 of 97 total).
Anopheles gambiae genome, the Spp27 homologs are found in eukaryotic genomes that do not have a clearly identifiable UBF.

The upstream rDNA promoter domain is recognized in a species-specific manner: the S.cerevisiae upstream rDNA promoter domain does not mediate any increase in rRNA synthesis when 5′ to either an S.cerevisiae or S.pombe rDNA core promoter domain in S.pombe in vitro transcription reactions. The possibility that different spacing between the upstream promoter and the core domains in the S.cerevisiae and S.pombe hybrid rDNA templates would increase their transcriptional efficiency, as was seen in the case of a Xenopus rDNA template in a heterologous murine transcription system (42), remains to be tested. While the S.pombe upstream rDNA promoter domain mediates a modest increase in rRNA synthesis in S.cerevisiae transcription reactions, it is critical for activating rRNA synthesis directed from the S.cerevisiae core rDNA promoter in in vitro S.pombe transcription reactions. Despite the homology observed between SpRrn5h and S.cerevisiae Rrn5p, and between SpRrn10h and S.cerevisiae Rrn10p, the S.pombe factors do not complement an S.cerevisiae rrn5- or rrn10- mutant strain, respectively (M.Tripp, K.van den Heuvel and L.Pape, data not shown). For SpRrn10h, this may be due to its inability to interact with S.cerevisiae Rrn9p (Fig. 6), in contrast to the interaction between S.cerevisiae Rrn10p and Rrn9p (34).

Recent studies point to nucleolar histone modification and chromatin remodel activity playing an important role in regulation of eukaryotic rRNA synthesis. Histone modifications and DNA methylation were shown to play a crucial role in activation of rRNA genes in hybrid plants (43,44). A histone acetyltransferase activity was found to co-purify with Xenopus RNA pol I (45), and mammalian rDNA transcription was shown to increase in vivo and in vitro when histone deacetylase was inhibited (12). Furthermore, a nucleolar, chromatin remodel activity that associates with the RNA pol I termination factor, TTF-I, was characterized in mammals (46), which aids in repressing rRNA synthesis by targeting histone deacetylase (47). The RSC nucleosome remodel complex has been found to target RNA polymerase III transcribed promoters as well as its pol II targeted promoters (48). The hypothesis that fungal UAF complexes are RNA pol I specific chromatin remodel factors, suggested based on the homology several subunits share with those of the SWI/SNF complex, can now be molecularly tested.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

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