Characterization of an *SNR* Gene Locus in *Saccharomyces cerevisiae* That Specifies Both Dispensible and Essential Small Nuclear RNAs

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A genetic locus is described that specifies two *Saccharomyces cerevisiae* small nuclear RNAs (snRNAs). The genes specifying the two snRNAs are separated by only 67 base pairs and are transcribed in the same direction. The product RNAs contain 128 and 190 nucleotides and are designated snR128 and snR190, respectively. These RNAs resemble snRNAs of other eucaryotes in nuclear localization and possession of a 5' trimethylguanosine cap. Neither snRNA is related in sequence to previously described vertebrate or yeast snRNAs. Both RNAs exhibit properties consistent with nucleolar organization and hydrogen bonding to pre-rRNA species, suggesting possible roles in ribosome biogenesis. The snR128 species cosediments with deproteinized 27S pre-rRNA, whereas snR190 is associated with a 20S intermediate. Gene disruption in vitro followed by replacement of the chromosomal alleles reveals that *SNR128* is essential, whereas *SNR190* is not.

The small nuclear RNAs (snRNAs) are a class of metabolically stable RNAs found in the nuclei of all eucaryotic organisms examined to date (for a review, see reference 9). The ubiquity of the snRNAs argues that they perform vital functions that have been conserved through evolution. Several lines of evidence indicate that at least six of the uridine-rich metazoan snRNAs participate in mRNA maturation; U1, U2, U4, U5, and U6 are implicated in intron splicing (3-5, 15, 16), whereas U7 is required for 3' editing of histone mRNAs (42, 43). Another snRNA, U3, is implicated in the processing of pre-rRNA (29, 51).

Recently, snRNAs have also been found in the yeast *Saccharomyces cerevisiae*, opening the way for detailed genetic analysis of these molecules (33, 49). Although several of the estimated two dozen yeast snRNAs have been shown to be dispensible, at least 5 are known to be essential for cell viability (see reference 33 for a summary; J. Zagorski and M. J. Fournier, unpublished observations). Each of the essential species appears to be analogous to a U RNA required for mammalian RNA processing. The yeast counterparts were identified from clear sequence homologies and physical association with the spliceosome. Yeast species snR19, Lsr1 (also known as snR20), snR14, and snR7 are analogous to the metazoan U1, U2, U4, and U5 species, respectively (2, 17, 25, 39, 40), whereas snR17 is related to the U3 snRNA (12). The snR7 and snR19 species are required for pre-mRNA splicing (17, 25) and mutations in Lsr1 can suppress otherwise nonfunctional mutations in the "TACTAAC" domain in yeast introns (24).

We describe here properties of two novel snRNA species from *S. cerevisiae*. These RNAs, designated snR128 and snR190, contain 128 and 190 nucleotides and are encoded by two tightly linked genes, *SNR128* and *SNR190*, respectively. Gene disruption and tetrad analyses have revealed that *SNR128* is essential for growth, whereas *SNR190* is dispensible. This brings to six the number of essential snRNA genes found in *S. cerevisiae*. Both snRNA species appear to be localized to the nucleolus and both are associated with

precursors to rRNA, suggesting possible roles for these molecules in ribosome biogenesis.

MATERIALS AND METHODS

Strains and culture conditions. Yeast strain A364A (a *ade1 ade2 ura1 his7 lys2 tyr1 gal1*) was the source of RNA. *SNR* DNA was from a λ Charon 4A bank of genomic DNA from the same strain. Gene replacement analyses were carried out with the diploid strains YNN290 (a/ α *ura3-52/ura3-52 trp1 Δ /trp1 Δ ade2-101/ade2-101 lys2-901/lys2-901*) and KY119 (a/ α *ura3-52/ura3-52 trp1 Δ /trp1 Δ ade2-101/ade2-101 lys2-901/lys2-901 his3 Δ /his3 Δ). Strains were maintained on rich medium containing glucose (YPD; 1% yeast extract, 2% peptone, 2% dextrose) or on minimal medium (SD; 0.67% yeast nitrogen base without amino acids, 2% dextrose) as described by Sherman et al. (38). Cells were incubated at 32°C unless otherwise indicated. Yeast transformations were done by using the lithium acetate procedure of Ito et al. (13). DNA from *S. cerevisiae* was prepared as described by Petes et al. (26), and RNA was prepared by the procedure of Sherman et al. (38). All other yeast manipulations were carried out as described by Sherman et al. (38).*

S1 nuclease analysis. DNAs were 3' end labeled with [α -³²P]dATP and DNA polymerase Klenow fragment (New England Biolabs, Inc.) or 5' end labeled with [γ -³²P]ATP and polynucleotide kinase (New England BioLabs) (22). RNA-DNA hybrids were formed by overnight incubation at 60°C with 60 μ g of total yeast RNA in 10 μ l of 80% formamide-3 $\times$ SSC (1 $\times$ SSC is 150 mM sodium chloride plus 15 mM sodium citrate [pH 7]). Hybridization was terminated by addition of 300 μ l of cold 250 mM sodium chloride, 30 mM sodium acetate (pH 4.5), and 4.5 mM zinc acetate. The sample was then divided into aliquots and digested for 30 min at room temperature with 0, 500, 2,500 or 5,000 U of S1 nuclease (Boehringer Mannheim). Digestion was terminated by addition of 1/4 volume of 2.5 M ammonium acetate-50 mM EDTA and precipitation with 2.5 volumes of ethanol. Following centrifugation, pellets were resuspended in 10 μ l of 80% formamide and 1 $\times$ TBE (89 mM Tris-borate [pH 8.3], 4

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mM EDTA) and fractionated through thin (0.5-mm) sequencing gels containing 6% polyacrylamide, 50% urea, and 1× TBE.

5' labeling of snRNAs. snRNAs were 5' labeled with polynucleotide kinase following partial alkaline digestion. RNA samples were suspended in 10 μ l of 100 mM Tris (pH 9.5) and incubated at 65°C for 15 min. An equal volume of 20 mM MgCl₂ was added, and the RNA was labeled for 1 h by incubation at 37°C with 10 U of polynucleotide kinase and 100 μ Ci of [γ -³²P]ATP.

Hybrid selection of RNAs. RNA was hybrid selected from total RNA by the method of Kafatos et al. (14) with the modifications described below. Plasmid DNA was restricted, denatured in 0.3 M sodium hydroxide, and neutralized with 1 volume of 2 M ammonium acetate. The DNA was then applied by suction to a 20-mm-diameter wet nitrocellulose filter (pore size, 0.45 μ m) preequilibrated in 10× SSC. The filter was washed with 2× SSC, air dried, and baked at 80°C for 1 h. The filter was then rewetted with water and incubated overnight with RNA in 50% formamide–4× SSC–0.1% sodium dodecyl sulfate at 37°C. Nonspecifically bound RNA was removed with two low-stringency washes (2× SSC plus 0.1% sodium dodecyl sulfate at room temperature) and two medium-stringency washes (0.1× SSC plus 0.1% sodium dodecyl sulfate at room temperature). Hybrid-selected RNA was removed by boiling the filter in water for 10 min.

Immunoprecipitation of snRNAs with trimethylguanosine (TMG) antibodies. Capped RNAs were precipitated by the method described by Adams et al. (1). RNAs and antibody were combined in phosphate-buffered saline (10 mM sodium phosphate [pH 7.5], 150 mM sodium chloride) at a ratio of 1 μ g of antibody per μ g of RNA and incubated on ice for 60 min. RNA-antibody complexes were collected with phosphate-buffered saline-washed Pansorbin (1 μ l/1 μ g of antibody; Calbiochem-Behring). After 30 min on ice, the immunoprecipitates were pelleted by centrifugation and washed three times with low-salt NET (50 mM Tris hydrochloride [pH 7.5], 150 mM sodium chloride, 5 mM EDTA) and twice with high-salt NET (containing 400 mM sodium chloride). The final pellet was suspended in 100 μ l of high-salt NET, extracted twice with phenol, extracted once with chloroform, and then ethanol precipitated.

Cell fractionation. Nuclei were purified as described by Hughes et al. (12). RNA was extracted by shaking at 4°C in buffer containing 20 mM HEPES (pH 7.9), 150 mM KCl, 10 mM MgCl₂, 10 mM dithiothreitol, 10 mM vanadyl ribonucleoside complex, and 0.2 mM phenylmethylsulfonyl fluoride or by sonication in the same buffer. Nuclear debris were pelleted by centrifugation at 14,000 $\times$ g for 10 min. Nondenaturing extraction of RNA and sucrose gradient fractionation were performed as described by Tollervey (45).

RESULTS

Identification of nuclear-enriched RNAs. The two snRNA genes described here were derived from a bank of cloned genes that specify nuclear-enriched small RNAs. The putative *SNR* bank was derived from a genomic library, in bacteriophage λ Charon 4A (6), by hybridization screening with a mixture of individually isolated RNAs prepared from highly purified nuclei. The probe RNAs consisted of 12 to 14 species that are very evidently enriched in the nucleus. Nuclear enrichment was demonstrated by comparing the electrophoretic patterns of nuclear and whole-cell small RNA (J. R. Thompson, J. Zagorski, J. L. Woolford, and

FIG. 1. TMG cap analysis of hybrid-selected snR128 and snR190. A nitrocellulose filter containing bound pJZ14 DNA was used to hybrid select snR128 and snR190 from whole-cell RNA. RNAs were then 3' end labeled with [³²P]pCp and RNA ligase (10) and incubated with anti-TMG antibody as described by Adams et al. (1). Lanes: 1, nuclear RNA, anti-TMG supernatant; 2, hybrid-selected RNA, anti-TMG supernatant; 3, nuclear RNA, anti-TMG immunoprecipitate; 4, hybrid-selected RNA, anti-TMG immunoprecipitate.

M. J. Fournier, *Nucleic Acids Res.*, in press). Our attention was focused early on one clone, λ JT4, after hybrid selection assays revealed that it actually specifies two RNAs. The two genes are very tightly linked and are wholly contained on a 1.5-kilobase-pair (kb) *Hind*III fragment from λ JT4. S1 nuclease mapping results described below demonstrate that these two molecules are 128 and 190 nucleotides in length. We refer to the RNAs as snR128 and snR190, respectively, and designate the corresponding genes as *SNR128* and *SNR190*.

TMG cap analysis. A recognized characteristic of metazoan snRNAs is the presence of a TMG cap (8, 21). To test for the presence of TMG caps on snR128 and snR190, we used a plasmid encoding both snRNAs to hybrid select these species from total cell RNA. The plasmid used for this and subsequent experiments (pJZ14) contains the 1.5-kb *Hind*III fragment from phage λ JT4 subcloned into pBR322. The hybrid-selected RNA was then incubated with TMG antibody to collect capped species. A gel profile of the resulting enriched species is shown in Fig. 1. The pattern of immunoprecipitated RNAs is shown in lane 4. Only two RNA species, corresponding in size to snR190 and snR128, are observed. The pattern of filter-bound RNAs is shown in lane 2. Numerous other RNA species, predominantly rRNAs and tRNAs, are also present as background; however, there is clear enrichment of the snR190 and snR128 species. The observation that only snR128 and snR190 were hybrid se-

FIG. 2. Nuclear extraction of snRNAs. Lanes: 1, total nuclear RNA; 2, RNA retained in nuclei following incubation in 150 mM KCl; 3, RNA released from nuclei with 150 mM KCl; 4, RNA from an in vitro splicing extract prepared as described by Lin et al. (20). Lanes 2 and 3 contain equivalent proportions of the same preparation. The positions of snR128, snR190, snR7S, and snR7L are indicated.

lected and immunoprecipitated (lane 4) demonstrates that the two snRNAs encoded by pJZ14 are TMG capped.

Subcellular condition of snR128 and snR190. We have examined the subnuclear localization of the snR128 and snR190 species by analyzing the RNA profiles for intact nuclei and nuclei extracted with salt or subjected to sonication. The salt extraction results shown, as Northern (RNA) blots in Fig. 2, suggest that both snRNAs occur in the nucleolus. At moderate salt concentrations (150 mM KCl) both snRNAs are retained in the nuclear fraction much more efficiently than a number of nucleoplasmic RNAs, e.g., pre-tRNA₃^{Lcu}, *CYH2* pre-mRNA (45), or the spliceosome-associated snRNAs snR7S, snR7L (Fig. 2), or Lsr1 (data not shown). Incubation of nuclei in 150 mM KCl releases the spliceosomal snR7 RNAs (Fig. 2, lane 3), while snR128 and snR190 are retained (lane 2). In addition, even sonication of isolated nuclei inefficiently release snR128 and snR190 (data not shown), a property characteristic of nucleolar RNAs in vertebrate cells (32).

The inferred nucleolar localization of snR128 and snR190 suggests a possible role in ribosome biogenesis, potentially related to rRNA maturation. To determine whether snR128 or snR190 are associated with precursors to rRNA, we conducted fractionation and hybridization analyses with total RNA prepared under conditions expected to disrupt protein-RNA interactions but to maintain RNA-RNA hydrogen bonding. Sphereoplasts were lysed and treated with 2% sodium dodecyl sulfate and 2 mg of proteinase K per ml at 14°C, and the cleared lysate was fractionated on a nondenaturing sucrose gradient. The distribution of high-molecular-weight rRNA and pre-rRNA species is shown in Fig. 3A. The pattern of low-molecular-weight rRNA, tRNA, and snRNA species is shown in Fig. 3B. Northern blots show peaks of snR128 and snR190 sedimenting lower in the gradient (i.e., of higher molecular weight) than expected either for free snRNAs or for small nuclear ribonucleoprotein particles. The high-molecular-weight peak of snR190 sediments with 20S pre-rRNA (Fig. 3A and B, lanes 4 to 6). snR128 sediments at approximately 27S (Fig. 3A and B, lanes 7 and 8), consistent with an association with either 27SA or 27SB pre-rRNA. Also shown in Fig. 3 is the distribution of snR17, which shows primary and secondary sequence homology to vertebrate U3 RNA (12) and is associated with 35S pre-rRNA. Neither the spliceosome-associated snRNA, snR7, nor tRNA₃^{Lcu} or its precursors show a tendency to sediment more rapidly than the free RNA. The distribution of the rRNA species is consistent with what is expected for protein-free RNA. However, even in the event that some protease- and detergent-resistant peptide remains associated with the snRNAs, the distribu-

tion patterns still require that they be associated with high-molecular-weight structures, since snRNPs sediment at only 10S.

Sequence of the *SNR190* and *SNR128* genes. The region of DNA corresponding to the *SNR190* and *SNR128* genes was sequenced by the M13 dideoxy method (36). Restriction fragments used in the sequencing were isolated from the 1.5-kb *SNR190-SNR128* subclone pJZ14. The 400-base-pair (bp) *Bgl*III-*Eco*RI fragment and the 350-bp *Bgl*III-*Nhe*I fragment from pJZ14 were sequenced in M13 in both orientations. The *Bgl*III junction of the two fragments was verified by sequencing an additional template that spanned this site by using a primer complementary to a region of the *SNR190* sequence. The sequence of the *SNR190-SNR128* gene region and the sequencing strategy are shown in Fig. 4.

Transcript mapping. A crude *SNR190* transcript map was generated by restricting pJZ14 with various single enzymes and combinations of enzymes, blotting the gel to nitrocellulose, and probing with 5'-labeled, gel-purified snR190 (data not shown). The pattern of hybridization observed allowed the snR190 transcript to be placed roughly between the *Bgl*III and *Dra*I sites indicated in Fig. 4B. To accurately locate the *SNR190* coding sequence, we mapped the gene by using S1 nuclease. The 3' end of the gene was mapped from the *Hinf*I site shown in Fig. 4A. *Hinf*I-digested pJZ14 was 3' end labeled with [α -³²P]dATP and digested with *Nhe*I, and a resulting 475-nucleotide *Hinf*I-*Nhe*I fragment was gel purified. This DNA was hybridized to total cell RNA and digested with S1 nuclease. The 5' end of the gene was mapped from the *Taq*I site shown in Fig. 4A. The 130-bp *Taq*I fragment from pJZ14 was gel purified, end labeled with [γ -³²P]ATP, hybridized to whole-cell RNA, and digested with S1 nuclease. The results of these analyses are shown in Fig. 5. A 151-nucleotide 3'-end-labeled fragment and a 44-nucleotide 5'-end-labeled fragment were protected from digestion. Taken together, and correcting for the overlap between the two restriction sites and the two bases added during the 3' end labeling, these data place the mature snR190 transcript on the DNA sequence as indicated in Fig. 4A. Because of the imprecision inherent in the S1 procedure, a size accuracy of plus or minus 1 to 2 nucleotides is assumed for each fragment. The sequence specifying the predicted mature RNA is indicated in italics and underlined.

The location of *SNR128* was also defined initially by Southern blot analysis as described for *SNR190*. The results of the hybridization analysis indicated that the gene overlapped the unique *Bst*EII site indicated in Fig. 4. The gene was mapped from this site by using S1 nuclease. *Bst*EII-digested pJZ14 was 5' labeled or 3' labeled and redigested with *Eco*RI. End-labeled *Bst*EII-*Eco*RI restriction fragments were gel purified, hybridized to whole-cell RNA, and digested with S1 nuclease. The results are shown in Fig. 6. Whole-cell RNA protected a 90-nucleotide 5'-end-labeled fragment and a 43-nucleotide 3'-end-labeled fragment. The predicted size of the mature transcript is the sum of these two numbers, or 133 nucleotides. However, since the 3' end labeling added 5 nucleotides to the end of this fragment, the actual size of the mature transcript is 128 nucleotides. The sequence corresponding to the mature RNA is indicated in italics and underlined. As above an error probability of plus or minus 1 to 2 nucleotides is assumed for each protected fragment. The directions of transcription of the snRNAs, as deduced from the S1 analysis, are indicated in Fig. 4B by arrows.

Sequence analysis. The deduced sequences of snR190 and snR128 were compared with those of other known snRNAs

FIG. 3. Distribution of RNA on nondenaturing sucrose gradients. Total RNA was isolated and deproteinized under nondenaturing conditions and centrifuged through a sucrose gradient as described by Tollervey (45). RNA was prepared from gradient fractions under denaturing conditions. (A) High-molecular-weight RNA separated on a 1.2% agarose-formaldehyde gel and transferred to nitrocellulose. The upper panel shows the distribution of the large rRNAs visualized by UV irradiation of the filter following Northern transfer. The lower panel shows the distribution of a number of pre-rRNA species detected by hybridization with a probe to internal transcribed spacer 1. (B) Low-molecular-weight RNA separated on an 8% polyacrylamide gel containing 8.3 M urea and transferred to a Hy-Bond membrane (Amersham Corp.). The upper panel shows the distribution of 5.8S, 5S, and tRNA, visualized by UV irradiation of the filter following Northern transfer. The lower panel shows the distribution of snR128, snR190, and snR17, as well as tRNA₃^{Leu} and its precursors, detected by hybridization with the cloned genes.

by using the sequence analysis package of Pustell and Kafatos (30). The RNAs examined included human U1, Novikoff hepatoma U2, U3, U4, U5, and U6, sea urchin U7, and human U8 RNAs (9, 31, 43). No significant relatedness was detected between these sequences and snR128 or snR190. In addition, neither snRNA corresponds to other sequenced yeast snRNAs. Solely on the basis of size, snR128 may correspond to the ca. 125-nucleotide snRNA designated snR13 by Wise et al. (49). As for snR190, several snRNAs similar in size have been described (33, 48, 49) but only one sequence has been reported (48). This RNA, snR3, does not correspond to snR190.

Further analysis of the DNA sequence revealed that snR190 contains two Sm antigen consensus binding sites (shown by asterisks in Fig. 4A). The Sm antigen is one of several known metazoan snRNA-binding proteins which assemble with snRNAs into small nuclear ribonucleoprotein particles. Anti-Sm antiserum capable of immunoprecipitat-

ing small nuclear ribonucleoprotein particles is produced by human patients with the autoimmune disease lupus erythematosus (19). In metazoans the Sm consensus sequence is AU₍₃₋₆₎G (7, 23). The homologous domains in snR190 are found at positions 88 to 93 (AUUUUG) and at positions 69 to 73 (AUUUG). The snR128 sequence was also examined for the presence of the consensus Sm binding site, but no such domain was found.

The sequences of *SNR190* and *SNR128* were also searched for the presence of yeast RNA polymerase II transcription initiation-termination signals (37, 50). A possible "TATAAA"-like transcription initiation sequence (TAT CAAA) was observed upstream of *SNR128* at positions -41 to -47, and polymerase II-like "TAGT...TTT" termination sequences were found at positions +75 to +78 and +98 to +100. Termination sequences for yeast polymerase II transcripts are typically found 10 to 40 nucleotides upstream of the polyadenylation site (50). The locations of the candi-

FIG. 4. Sequence of the *SNR190-SNR128* locus and flanking regions. The sequence shown (A) was obtained from an *EcoRI-NheI* fragment of pJZ14 by using the M13 dideoxy method of Sanger et al. (36). The structure of the fragment and sequencing strategy are illustrated (B), and restriction sites used in the S1 analyses are also indicated. The snR190 and snR128 coding regions are highlighted by italics and underlines. Sequences corresponding to the consensus Sm antigen-binding domain are indicated by asterisks. Transcriptional orientations of snR190 and snR128 are indicated by arrows.

date *SNR128* signals are consistent with the consensus data. In contrast, *SNR190* has a potential TATAAA site upstream at positions -20 to -25 (TTTAAA), but no good polymerase II termination sequence. By analogy to vertebrate snRNAs, it is generally assumed that *S. cerevisiae* snRNAs are polymerase II transcripts. However, this has not been experimentally determined for any yeast snRNA, including snR128 and snR190.

***SNR190* is a dispensible gene.** To determine the essentiality of *SNR190*, we transplanted a nonfunctional allele of *SNR190* into the genome of a diploid host. Heterozygous transformants were then examined by tetrad analysis. The strategy is shown in Fig. 7A. An 850-bp *EcoRI-BglII* fragment containing the yeast *TRP1* gene was used to replace the ca. 350-bp *EcoRI-BglII* fragment of pJZ14, which contains the *SNR190* coding region. The 1.9-kb *ClaI* fragment from the resulting plasmid, pJZ26, was then used to transform the *trp* diploid YNN290 to tryptophan prototrophy by the method of Rothstein (35). DNA from seven *trp*⁺ transformants was screened for *SNR190/snr190::TRP1* heterozygotes by Southern blot analysis. The results of the analysis are shown in

FIG. 5. S1 nuclease mapping of *SNR190*. Abbreviations: 5'-DNA, 5'-end-labeled *TaqI* restriction fragment; 3'-DNA, 3'-end-labeled *HinfI* restriction fragment. Lanes: 0, no S1 nuclease; 1, 500 U of S1; 5, 2,500 U of S1; S, *Sau3A* digest of pBR322 (molecular weight markers); L, M13 dideoxy sequencing ladder. DNA fragments protected from S1 nuclease digestion are indicated by arrows.

Fig. 7B. Six of the seven transformants analyzed yielded hybridizing *HindIII* fragments characteristic of the desired *SNR190/snr190::TRP1* heterozygote, i.e., a 1.5-kb fragment (*SNR190*) and 1.1 and 0.8-kb fragments (*snr190::TRP1*). Transformant YNN(*TRP*)3 appeared to be homozygous for *snr190::TRP1*. The isolation of this apparent homozygote strongly indicated that *SNR190* is dispensible.

Transformants YNN(*TRP*)1, YNN(*TRP*)2, and YNN(*TRP*)3, as well as the positive control YNN290, were sporulated and tetrads were dissected to specifically assess the essentiality of *SNR190*. Nineteen tetrads each from strains YNN(*TRP*)1 and YNN(*TRP*)2 were dissected. The majority of tetrads examined (33 of 38) yielded four viable spores, demonstrating that *SNR190* is dispensible (data not shown). The segregation of the *TRP1* marker was 2+:2- in all four-spored asci scored. The results for YNN(*TRP*)3 were also consistent with the Southern blot analysis, showing that this strain is a *snr190::TRP1* homozygote. All tetrads examined yielded four viable *trp*⁺ spores each (data not shown).

The dispensibility of snR190 was verified by a Northern blot analysis of total RNA from the four haploids resulting from a YNN(*TRP*)2 tetrad. No snR190 was detected in the

FIG. 6. S1 nuclease mapping of *SNR128*. (A) 3'-end-labeled *BstEII*-digested pJZ14. Lanes: 1 to 3, 5,000, 2,500, and 500 U of S1 nuclease, respectively; 4, *Sau3A* digest of pBR322 as molecular weight markers; 5, no S1 nuclease. (B) 5'-end-labeled *BstEII*-digested pJZ14. Lanes: 1, no S1 nuclease; 2, 2,500 U of S1; 3, *HaeIII* digest of pBR322. (C) 5'-end-labeled *BstEII*-digested pJZ14 reanalyzed as described for panel B. The sizes of the *Sau3A* and *AluI* restriction fragments in the size range of the protected fragments are given to the right of each panel. Protected fragments are identified by arrows.

trp⁺ haploids YNN(*TRP*)2-15A and YNN(*TRP*)2-15B (Fig. 8A, lanes 1 and 2). This result confirms that snR190 is absent in cells containing the *snr190::TRP1* allele. Also note that snR128 accumulates to a lesser extent in these strains. The reduced levels of snR128 in the *snr190* strains were verified by performing a second Northern blot analysis (Fig. 8B; compare lanes 5 and 8 for *snr190* and *snr190*⁺ strains, respectively). This suggests that the replacement of *SNR190* may have compromised the activity of the *SNR128* promoter, which is a surprising result since the replacement deleted little *SNR190-SNR128* intergenic DNA. It is unlikely that the replacement of *SNR190* with *TRP1* deleted more intergenic DNA than expected. The wild-type DNA sequence contains only one *BglII* site in this region, namely, that used for the gene replacement.

***SNR128* is essential for growth.** *SNR128* was tested for essentiality by the strategy indicated in Fig. 9A. The *BstEII* site in pJZ14 was first converted to *BamHI* with linkers, and a 1.8-kb *BamHI* fragment containing the yeast *HIS3* gene was then inserted. A *ClaI* digest of the resulting plasmid, pJZ30, was used to transform the *his* diploid KY119 to

FIG. 7. (A) Structure of the *SNR190* locus and gene replacement strategy. Plasmids containing the wild-type and *snr190::TRP1* alleles, pJZ14 and pJZ26, are shown. (B) Identification of *SNR190/snr190::TRP1* heterozygotes by Southern blot analysis of *HindIII*-digested genomic DNAs. The *SNR190* probe was the 1.5-kb *HindIII* fragment from pJZ14. Lanes: 1, DNA from YNN290; 2 through 8, DNAs from *trp*⁺ transformants YNN(*TRP*)1 through YNN(*TRP*)7, respectively. Hybridizing *HindIII* fragments of the size expected for the *snr190::TRP1* allele are indicated by asterisks.

histidine prototrophy. *his*⁺ transformants were screened for the correct structure by Southern blot analysis. All seven transformants screened gave rise to restriction fragments characteristic of both the *SNR128* allele (a 1.3-kb *ClaI* fragment) and the *snr128::HIS3* allele (2.3- and 0.8-kb *ClaI* fragments) (Fig. 9B). The host strain KY119 yielded only the *SNR128* fragment.

Four heterozygous strains, KY(*HIS*)1, KY(*HIS*)2, KY(*HIS*)3, and KY(*HIS*)4, were sporulated and dissected to determine the essentiality of *SNR128*. Ten tetrads from each strain were dissected. All four strains gave rise to tetrads yielding predominantly two viable spores (Fig. 10). All 16 viable spores from eight tetrads examined were *his*. This pattern of viability and segregation of the selectable marker is consistent with *SNR128* being an essential gene.

The possibility that the lethality of the *HIS3* insertion is a polar effect on another gene and not a direct result of *SNR128* disruption can be excluded. We have fused *SNR128*

FIG. 8. Northern blot analysis of snR190 in cells containing a disrupted *SNR190* gene. (A) The blot shown is for total small RNA from control and test cells fractionated in a 7% polyacrylamide gel containing 7 M urea. The gel was transferred electrophoretically to a Zeta-Probe nylon membrane (Bio-Rad Laboratories) and probed for snR190 and snR128. The *SNR190* probe was the 1.5-kb *HindIII* fragment from pJZ14. Lane N, 3'-end-labeled yeast nuclear RNA standard; lanes 1 through 4, haploids YNN(*TRP*)2-15 A through YNN(*TRP*)2-15 D, respectively. The positions of snR190 and the 5S and 5.8S rRNAs are indicated. (B) Nitrocellulose Northern blot of RNAs separated in a 1.5% agarose gel containing 17% formaldehyde and probed as described above. Lanes 5 and 8 compare snR190 and snR128 levels in *trp*⁺ and *trp* strains, respectively. Lanes 6 and 7 contain RNA from the same strain as in lane 5, but obtained under heat shock growth conditions as part of a different analysis.

to the repressible *GAL*I upstream activation sequence (11) and constructed a haploid strain containing the *GAL*I::*SNR128* fusion as the sole *SNR128* allele (J. Zagorski, H.-D. Li, and M. J. Fournier, manuscript in preparation). This strain is galactose dependent and will grow on glucose only if snR128 is supplied from a plasmid. A ca. 700-bp *NheI*-*EcoRI* segment (Fig. 4B) that is mostly *SNR128* and dispensable *SNR190* DNA is sufficient for complementation when present on a multicopy plasmid (data not shown).

The level of snR128 required to support normal growth has not been accurately determined. However, *snr190::TRP1* strains, which have approximately 10-fold less snR128 than wild-type strains do, are unimpaired in growth (Fig. 7, lanes 1 and 2). Furthermore, haploids inheriting the *snr128::HIS3* allele following sporulation of a heterozygous diploid were observed to undergo approximately three to five cell divisions (approximately 10 to 20 cells per microcolony). This result suggests that the minimum requirement for snR128 is perhaps 5 to 10% of the wild-type level, assuming simple dilution of the initial supply into daughter cells.

FIG. 9. *SNR128* gene disruption strategy and identification of *SNR128/snr128::HIS3* heterozygotes. Top: structures of the plasmids containing the wild-type and *snr128::HIS3* alleles. Bottom: *his*⁺ transformants were screened for the appropriate genomic structure by Southern blot analysis of *Clal*-digested DNAs. The hybridization probe was the 600-bp *TaqI* fragments from pJZ14 indicated in the figure. Lane C, KY119; lanes 1 to 8, transformants KY(*HIS*)1 through KY(*HIS*)8, respectively.

FIG. 10. Tetrad analysis of *SNR128/snr128::HIS3* heterozygotes. Diploids were sporulated, treated with Glusulase (Endo Laboratories), and dissected on a YPD agar slab. Following tetrad dissection, the agar slabs were mounted on YPD agar plates and incubated for 3 days at 32°C. Four panels are shown in the figure, each representing the results of dissecting 10 tetrads from four different *his*⁺ transformants. The transformants are designated KY(*HIS*)-1, KY(*HIS*)-2, KY(*HIS*)-3, and KY(*HIS*)-4. The apparent third viable spore resulting from the second tetrad of KY(*HIS*)1 is a nonyeast contaminant.

DISCUSSION

Of the essential *S. cerevisiae* snRNAs described to date, only snR128 lacks clear sequence relatedness to a vertebrate U snRNA. Consistent with this observation, several other groups have reported characterizations of apparent *S. cerevisiae* homologs of U1, U2, U4, and U5, i.e., snR19, Lsr1, snR14, and snR7, respectively (2, 17, 25, 39, 40). All of these snRNAs are found in the spliceosome (17, 27), and snR7, snR19, and Lsr1 have been shown to be involved in splicing. In addition, an apparent U6 homolog (snR6), was also identified during the characterization of snR14 (39). Thus, analogs for all of the vertebrate U snRNAs known to function in mRNA splicing seem to have been identified.

Interestingly, snR128 has structural features expected of a molecule involved in mRNA splicing. Examination of the snR128 sequence reveals two domains which show excellent complementarity to the two yeast mRNA splice site consensus sequences. In particular, snR128 nucleotides +30 to +35 (5'-ACAUUC-3') are complementary at five of six positions to the yeast mRNA intron 5' consensus sequence (5'-GUAUGU-3' [44]). Nucleotides +73 to +79 (5'-GUUA GUA-3') are complementary at all seven positions to the conserved TACTAAC box (5'-UACUAAC-3' [18, 28]). The presence of these sequence elements might raise the possibility that snR128 functions in *S. cerevisiae* as a U1 and/or U2 RNA analog. However, we have found no requirement for this snRNA species in splicing either *in vitro* (J. Zagorski, B. C. Rymond, M. Rosbash, and M. J. Fournier, unpublished observations) or *in vivo* (Zagorski et al., in preparation). Taken with the finding that more likely U1 (17) and U2 (2) candidates have been found in *S. cerevisiae*, these negative data suggest that the presence of the intron complementary domains may be fortuitous.

A number of other yeast snRNAs (snR3, snR4, snR6, snR8, snR9, snR10, and snR17) show properties consistent with hydrogen bonding to pre-rRNA species (45), and to these can now be added snR128 and snR190, which are associated with 27S and 20S rRNA, respectively. These nine snRNAs have other features in common with each other and with vertebrate U3, which is also hydrogen bonded to pre-rRNA (29, 51). Consistent with nucleolar localization, they are efficiently retained in the nucleus following extraction at moderate salt concentrations (150 mM) and, for this reason, are largely absent from *in vitro* splicing extracts. They are not detectably precipitated from yeast extracts by human anti-Sm sera (12, 47) or following injection into *Xenopus* oocytes (47) or eggs (34). Moreover, a human antinucleolar antibody has recently been identified which strongly precipitates snR128, snR190, and other yeast snRNAs of this class. (D. Tollervey and R. Luhrmann, unpublished observations), whereas the absence of snR10 interferes with the processing of pre-rRNA (45).

In contrast, the spliceosome-associated snRNAs of vertebrates or *S. cerevisiae* are readily released from the nucleus under the same conditions and are Sm precipitable. We interpret these data as indicating that *S. cerevisiae* contains at least nine snRNAs organized in snRNPs which exhibit structural and functional properties of the vertebrate U3 snRNA. This raises the possibility that other vertebrate snRNAs will be found associated with pre-rRNA, possibly including species with homology to snR128 and snR190. The actual roles played by the various pre-rRNA-associated snRNAs are unknown. It seems clear, however, that they have different functions in rRNA synthesis or ribosome assembly, since different phenotypes are observed in strains

lacking different snRNAs. The absence of snR190 or snR3 (48) has no detectable effect on growth, the absence of snR10 substantially impairs growth (46), whereas snR128 and snR17 (12) are essential for viability. Moreover, different snRNAs are associated with different pre-rRNA intermediates: snR3, snR4, snR10, and snR17 with 35S; snR128 with 27S; and snR190, snR5, and snR8 with 20S. The identification of a lethal phenotype associated with the *snr128::HIS3* genotype and the construction of a conditional allele, *SNR128*, under the control of the *GAL1* promoter offer the possibility of directly testing the role of this particular snRNA in ribosome biogenesis.

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