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Deletion of a yeast small nuclear RNA gene impairs growth

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We have cloned and sequenced the single copy gene *SNR10* which encodes the yeast small nuclear RNA, snR10. This species does not show obvious primary sequence homology to any previously identified small nuclear RNA. As an inital step towards determining the function of snR10, we have introduced insertions and deletions into the chromosomal copy of the gene. Strains lacking an intact copy of *SNR10* are viable but considerably impaired in growth, particularly at elevated osmotic strengths or low temperatures; at 25°C the doubling time of *snr10*$^-$ strains is 47% greater than that of otherwise isogenic *SNR10* strains. As judged by the incorporation of radioactive precursors, *snr10*$^-$ strains are impaired in net RNA synthesis at low temperatures. The identification of a leaky, conditional phenotype associated with the deletion of this small nuclear RNA gene was entirely unexpected since the defect in snR10 synthesis is complete and non-conditional.

Key words: snRNA/yeast/snRNA gene/gene disruption

Introduction

Small nuclear RNAs (snRNAs), which are contained in ribonucleoprotein particles (snRNPs) (reviwed by Busch *et al.*, 1982), are thought to play a role in a variety of RNA processing reactions. For example, the nucleolar species U3 is found associated with 28S pre-rRNA but not with the mature species (Prestayko *et al.*, 1970; Zieve and Penman, 1976) and specific molecular models have been proposed for the participation of U3 in the processing of pre-rRNA (Crouch *et al.*, 1983; Bachellerie *et al.*, 1983). The remaining U-snRNAs are nucleoplasmic, and all may be involved in the processing of pre-mRNA. The U1 snRNP has been shown to be required for splicing *in vitro* (Mount *et al.*, 1983; Padgett *et al.*, 1983; Kramer *et al.*, 1984) and in *Xenopus* oocytes (Fradin *et al.*, 1984). Recent experiments (Black *et al.*, 1985; Krainer and Maniatis, 1985) have now shown the additional requirement for U2 RNA in intron removal. Finally, the newly identified species U7 is required for the post-transcriptional cleavage of sea urchin histone H3 pre-mRNA in *Xenopus* oocytes (Galli *et al.*, 1983; Strub *et al.*, 1984; Georgiev and Birnstiel, 1985).

While a specific molecular model has been presented for the participation of U4 in transcription termination/polyadenylation (Berget, 1984), supporting experimental evidence has yet to be provided. Still less is known about the function of U5 or U6, although since the latter occupies the same snRNP particle as U4 (Hashimoto and Steitz, 1984; Bringmann *et al.*, 1984) it is likely that these species are involved in the same, or closely related processes.

Functional analyses have been severely hampered by the lack of tractable genetic systems in the higher eukaryotes, from which most information about snRNAs has been obtained. For this reason we initiated a study of snRNA function in the yeast *Saccharomyces cerevisiae*, which contains snRNAs encoded by single copy genes (Wise *et al.*, 1983). This finding is in marked contrast to the situation in higher eukaryotes and immediately offered the opportunity for using gene replacement techniques to test directly snRNA functions *in vivo*.

The first species to be analysed in this manner, designated snR3, has potential primary and secondary structural homology to mammalian U4 and U6 snRNAs (Tollervey *et al.*, 1983). Surprisingly, cells lacking an intact copy of the gene *SNR3* are not only viable but are indistinguishable from wild-type sister strains under all conditions tested, including growth competition in a chemostat. Here we report our analysis of a second yeast snRNA, snR10. Cells lacking an intact copy of the gene *SNR10* have a leaky cold-sensitive phenotype and are sensitive to elevated osmotic pressures. While these results demonstrate that snR10, unlike snR3, is required for wild-type growth, this conditional phenotype was completely unexpected, in that the inactivation of *SNR10* is entirely non-conditional.

Results

Sequence of SNR10

The cloning and initial characterisation of the single copy *SNR10* gene has been described (Wise *et al.*, 1983). The gene was originally isolated as a 5-kb genomic clone from a *Sau*3A partial bank in YEp13; Figure 1 shows a restriction map of the plasmid YEpR10. The approximate location of the coding sequence was first determined by Southern hybridisation using labelled cDNA to snR10. The DNA sequence was determined by di-deoxynucleotide sequencing (Sanger *et al.*, 1977) of fragments subcloned in M13 (Messing *et al.*, 1981). The end-points of the coding region were determined by direct enzymatic sequencing of purified snR10, labelled either at the 5′ or 3′ end. End-labelled RNA was generated from size-fractionated RNA eluted from a preparative 6% polyacrylamide gel containing 7 M urea; snR10 was purified on a two-dimensional polyacrylamide gel system as described previously (Wise *et al.*, 1983).

From these experiments we deduce that snR10 is 245 nucleotides in length. In addition to the major snR10 RNA, a less abundant species ~20 nucleotides longer can also be observed by Northern hybridisation (see Figure 4). The end-points of this species have not yet been mapped. However, a similar observation has been made for snR3 (Tollervey *et al.*, 1983); in this case the minor species is co-linear with the major RNA at the 3′ end. One plausible explanation is that these species arise as a result of heterogeneity in the site of transcription initiation, as has been observed for many other yeast genes (see, for example, Faye *et al.*, 1981; Bennetzen and Hall, 1982).

The complete nucleotide sequence of snR10 and *SNR10* together with the 5′- and 3′-flanking regions is shown in Figure 2. As is the case for the previously sequenced yeast snRNA gene *SNR3* (Tollervey *et al.*, 1983), the coding sequence is preceded

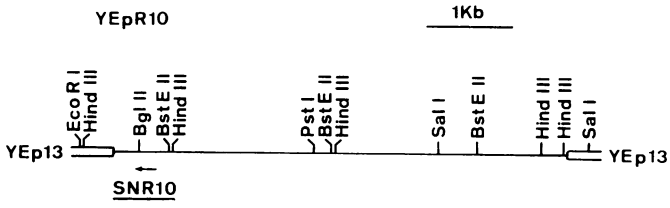

Fig. 1. Restriction map of YEpR10. This plasmid contains a 5-kb *Sau*3A genomic clone inserted into the *Bam*HI site of YEp13 (pBR322, *LEU2*, 2 μ) (Broach *et al.*, 1979). The location and orientation of the coding sequence of *SNR10* are indicated. The 1.05-kb *Hind*III fragment containing *SNR10* which was subcloned for use in subsequent experiments extends out of the insert into the pBR322 region of the vector.

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HindIII
|
AAGCTTCTTCTATAGCGTAGAGTAAGTAGTGCCTTGTAGCCCTACCGTTTTACAATGCAT

BstEII
|
GAGGTTACCCGCACTTATTATTTTTTTCTCTTTTTTTTTCTTAGCTATAAAAGGCAGATC


AATGCAGCATTCAATTGCTTTATTTGACTTTCCTTTACTATTTATTTACTTTCCATTTCT

                                                          RsaI
                                                 10       20       30 |
TATTTTAGTGTTATTTTATAACCAGTAACGCAAATTTAACAGCCATTCGTAACACGTACA
                      m2,2,7GpppAACGCAAAUUUAACAGCCAUUCGUAACACGUACA

    40        50        60        70        80        90
GTATCTCGTCGAGGTTGAACCCCTCCGGGGCGTTCTTGACCCATGAAGAATGTGGATTGG
GUAUAUCGUCGAGGUUGAACCCCUCCGGGGCGUUCUUGACCCAUGAAGAAUGUGGAUUGG
                                                  ClaI
   100       110       120       130       140|      150
TGTTGCAATATATTTATTATTGGCTTGGACTGGAGAACAAATTTATCGATCTTGGGTGCA
UGUUGCAAUAUAUUUAUUAUUGGCUUGGACUGGAGAACAAAUUUAUCGAUCUUGGGUGCA

                                Bgl II
   160       170       180       |190       200       210
ACAGTCTTTCTGTCGTCTGTTTTTTAGCAGATCTAAGGGTTTACCTTCGTGTGCCCGGAT
ACACUCUUUCUGUCGUCUGUUUUUUAGCAGAUCUAAGGGUUUACCUUCGUGUGCCCGGAU

   220       230       240
GAGGACCGTTGCAAGGATTGATAATACAACTATATACATCATTATCGTAGTGAATTGCAC
GAGGACCGUUGCAAGGAUUGAUAAUACAACUOH

GAGTAACTTCTCGGCATGGGGTGAGTAAATTCATATTATGAAAGTAATTAAACTTACTAC


CTAAATAAGAGGATATCTATGTCTTATTGCTTGTATATATGAGTGGCTTGGAATGCTGCA
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Fig. 2. DNA and RNA sequence of *SNR10*. The upper line shows the sequence of the non-coding DNA strand, beginning 206 nucleotides before the 5′ end of snR10 and extending 149 nucleotides beyond the 3′ end of the RNA. The lower line shows the sequence of the RNA together with the 5′ cap structure (Wise *et al.*, 1983). Sites of cleavage of restriction enzymes relevant to subsequent experiments are shown (the *Cla*I site can be cleaved only in DNA extracted from *DAM*$^-$ strains of *Escherichia coli*).

by a presumptive 'TATA' box (TATAAAAG) ~100 nucleotides 5′ to the start site, a position similar to that observed for other yeast genes transcribed by polymerase II (reviewed by Sentenac and Hall, 1982). No sequences resembling a consensus polymerase III promoter (DeFranco *et al.*, 1980; Sakonju *et al.*, 1980; Bogenhagen *et al.*, 1980; Fowlkes and Shenk, 1980) are found in *SNR10*. These results, together with studies on snRNAs in other systems (Jensen *et al.*, 1979; Murphy *et al.*, 1982) indicate that *SNR10* is transcribed by polymerase II. We can discern a region of homology (21/28) between *SNR3* and *SNR10* in the region of the 'TATA' box; sequences further upstream cannot be compared due to insufficient data for *SNR3*. Short sequences which mediate general amino acid control are repeated upstream from a number of co-regulated yeast genes (Donahue *et al.*, 1983; Hinnebusch and Fink, 1983). In this case, however, the significance is unclear since the homology is not conserved in other yeast snRNA genes (Tollervey, Simmons and Guthrie, unpublished; Patterson and Guthrie, in preparation). Interestingly, the initiating trinucleotide sequence AAC has been found at the encoded 5′ end of each of the five yeast snRNAs we have sequenced to date (*op cit.*).

No systematic attempt has been made to study the regulation of snR10 synthesis but, as judged by Northern blotting, the quantity of snR10 as a proportion of total RNA appears identical on minimal versus complete medium, on fermentable versus non-fermentable carbon sources and at growth temperatures of 25, 30 or 36°C.

The level of snR10 synthesised in a *snr10.3* strain carrying the low copy number plasmid YCpR10 (see below) is identical to that of *SNR10* strains suggesting that the information necessary for regulation lies within 210 nucleotides of the gene. A strain transformed with the high copy number plasmid YEpR10 contains ~20-fold more snR10 than does an isogenic strain lacking the plasmid, a value which is probably close to the average copy number of YEp13 (Futcher and Cox, 1984). The over-produced snR10 has the normal 5′ end and normal mobility on one- and two-dimensional gels. YEpR10 carries 4.5 kb of 5′-flanking DNA and 210 bp of 3′-flanking DNA, and it is thus likely that all the normal control regions are present. We conclude that *SNR10* is apparently not subject to feedback regulation. We have not detected any effect on growth resulting from the overproduction of snR10.

We have compared the sequence of snR10 with that of snRNAs from mammals, but in the absence of two-dimensional structure or other corroborative data, the alignments obtained do not provide compelling evidence for an evolutionary relationship between snR10 and a particular mammalian snRNA. Under conditions of reduced stringency, cross-hybridisation was observed to a small RNA species ~195 nucleotides in length from the filamentous fungus *Aspergillus nidulans*.

Gene replacements

To construct the insertions and deletions to be used for gene replacement experiments, a 1.05-kb *Hind*III fragment containing *SNR10* was subcloned into pUC9 (see Figure 3). This region contains 700 bp of yeast DNA together with the 346 bp of pBR322 DNA between the *Hind*III and *Bam*HI sites. Using this subclone, fragments containing the selectable marker *LEU2* were inserted into the coding sequence of *SNR10*; in some cases regions of *SNR10* were also deleted (see Figure 3). The three constructions shown are designated by their allele names, *snr10.1*, *snr10.2* and *snr10.3*. To replace precisely the chromosomal copy of *SNR10* with the copy disrupted *in vitro* (Rothstein, 1983), recipient strains of yeast carrying the double mutation *leu2-3, leu2-112* were transformed using linear fragments of DNA with selection for the inserted *LEU2* marker.

In each case it was found that the *Hind*III fragment carrying 346 bp of pBR322 DNA could be used successfully with this transformation technique. However, while insufficient transformants have been studied for statistical analysis, it appears that the frequency of transformation is reduced, and the proportion of transformants in which the introduced *LEU*$^+$ marker is linked to the *LEU2* locus is increased, relative to constructs in which both ends are homologous to the yeast chromosome. Presumably the pBR322 sequences are removed nucleolytically to reveal the yeast DNA; yet the success of the replacement is perhaps particularly surprising in the case of *snr10.3*, since in this construc-

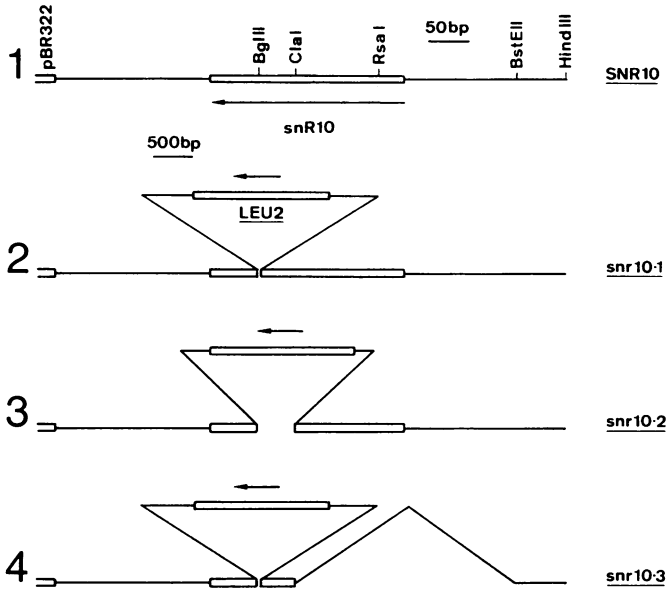

Fig. 3. Insertions and deletions made in *SNR10*. **(1)** Region containing *SNR10* which was subcloned into pUC9. **(2)** Construction 1: a 3-kb region containing the yeast selectable marker *LEU2* is inserted into the *Bgl*II site within the coding sequence of *SNR10*. **(3)** Construction 2: a 2.4-kb region containing *LEU2* is inserted between the *Bgl*II and *Cla*I sites resulting in the deletion of 40 bp of coding sequences. **(4)** Construction 3: a 3-kb region containing *LEU2* is inserted into the *Bgl*II site and the region between the *Cla*I and *Bst*EII sites is deleted, removing 140 bp of coding sequence and 138 bp of 5′-flanking sequence.

tion there are only 63 bp of DNA homologous to the *SNR10* locus at the non-pBR322 end of the linear fragment. Southern analysis shows that the correct constructions have been made and inserted in the yeast genome (data not shown).

Northern analysis (Figure 4) demonstrates that, in each case, the only copy of *SNR10* has been disrupted. However, while the initial construction, *snr10.1*, fully blocks the synthesis of snR10 during growth at either 25 or 36°C, this allele is associated with an apparent fusion transcript, the size of which (~510 nucleotides) is appropriate for a species which originates at the *SNR10* promoter and terminates at the transcription termination site in the fragment of the *TY1-17* element which is carried in the inserted *LEU2* region (Kingsman *et al.*, 1981; Casadaban *et al.*, 1983; Elder *et al.*, 1983). To eliminate the possibility that this fusion transcript, which probably includes the 5′ 195 nucleotides of snR10, is responsible for the residual growth of *snr10.1* strains, we constructed *snr10.2* and *snr10.3*. In RNA extracted from *snr10.2* strains, from which 40 bp of *SNR10* coding sequence have been deleted, a fusion transcript of ~600 nucleotides can be detected. If this species originates at the *SNR10* promoter it should terminate within the coding region of *LEU2*. The *snr10.3* construct deletes 140 bp of coding sequence and 138 bp of 5′-flanking DNA, including the 'TATA' box; in *snr10.3* strains we can detect neither fusion transcripts nor snR10 following growth at 25 or 36°C (Figure 4). We conclude that at least *snr10.3* is a genuine null allele.

Biological phenotype

Before performing the experiments just described, we first introduced *snr10.1* into one chromosome of a diploid strain to test for lethality. The growth of heterozygous diploids carrying a single intact copy of *SNR10* is indistinguishable from that of wild-type strains, demonstrating that the biological phenotype of *snr10.1* is fully recessive. When these transformants are sporulated, dissected and germinated at 30°C, only two spores per four spored ascus exhibit wild-type growth (Figure 5). This impaired growth is shown only by *LEU*$^+$ spores; Southern analysis (data not shown) confirms the segregation of this phenotype with the *snr10.1* allele. Subsequently, the *snr10.1*, *snr10.2* and *snr10.3* alleles were each introduced separately into the same haploid strain. In each case the resulting phenotype was indistinguishable from that of *snr10.1* segregants derived from transformed diploids.

On solid media at 36°C, *snr10*$^-$ strains cannot easily be distinguished from otherwise isogenic *SNR10* strains, however, a very marked difference in colony size is visible at 25°C. In li-

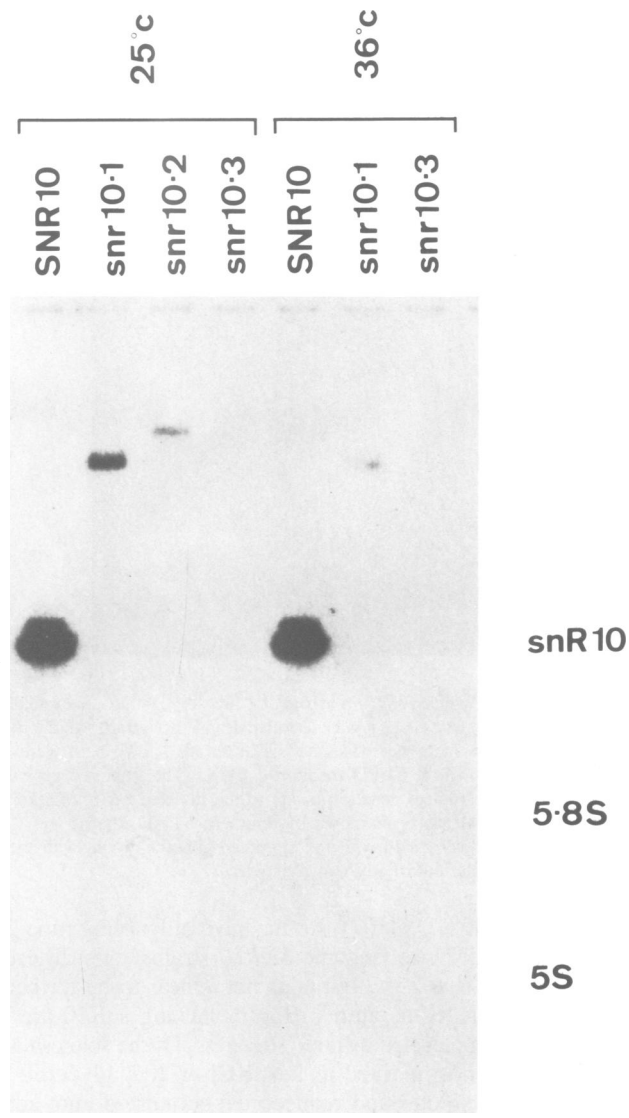

Fig. 4. Northern blot of strains carrying *SNR10* and *snr10.1*, *.2* or *.3*. RNA was prepared from strains which were isogenic except for carrying the *SNR10* allele indicated, following growth at the temperature indicated, and was electrophoresed on a 8% polyacrylamide gel and transferred to Genescreen. The probe for this blot is the 160-bp *Bgl*II-*Rsa*I fragment of *SNR10* which lies to the 5′ side of the *LEU2* insertion in each case (see Figure 3). Southern blots (data not shown) confirm that this probe hybridises to the region remaining in *snr10.2* and *snr10.3*, though less efficiently than with the intact gene. Northern blots probed with a 1.05-kb *Hind*III fragment which carries 210 bp of flanking DNA to each side of *SNR10* (see Figure 1) give similar results, as do blots made from agarose/formaldehyde gels, from which high mol. wt. RNA species transfer more efficiently than from acrylamide gels.

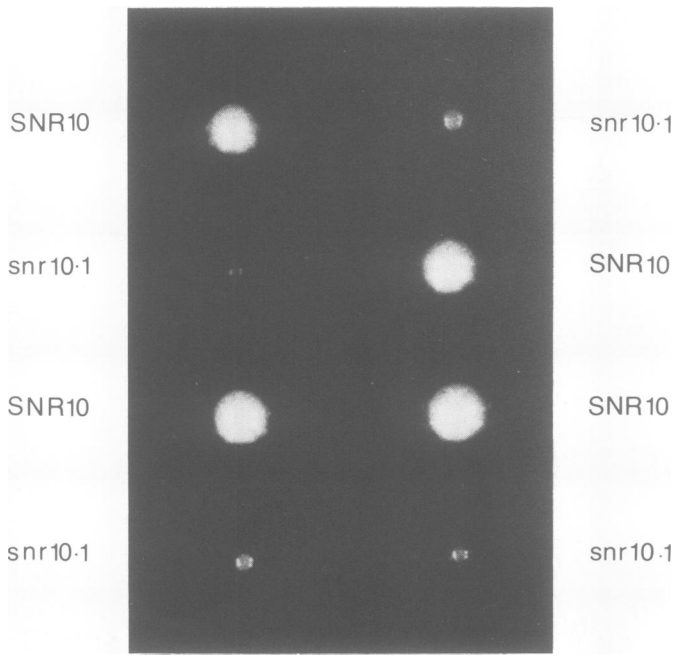

Fig. 5. Tetrads in which *SNR10* and *snr10.1* are segregating. A diploid strain heterozygous for *snr10.1* was sporulated and the tetrads which contain the four meiotic products were dissected. The tetrads shown here were germinated and grown on YEPD medium at 30°C. The impaired growth phenotype shows complete genetic linkage to the inserted LEU^+ marker in 25 tetrads tested and has been shown by Southern and Northern hybridisation to be associated with the disrupted gene copy and with the absence of detectable snR10 RNA in four tetrads.

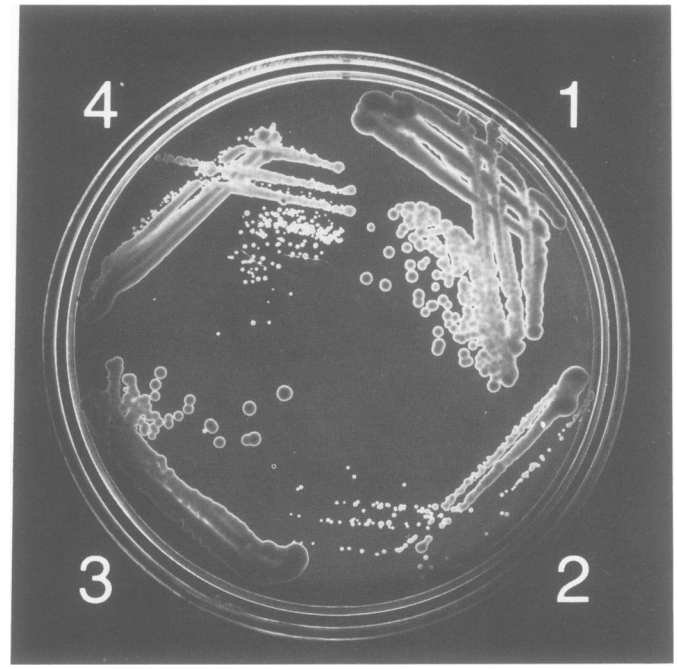

Fig. 6. *snr10.3* is complemented in *trans* by *SNR10*. The strains are shown growing on YEPD + 1 M KCl at 25°C. **(1)** *SNR10* strain. **(2)** *snr10.3* strain. The strain shown in sector 1 was transformed to *snr10.3*. **(3)** *snr10.3* strain carrying YCpR10. The strain shown in sector 2 was transformed with YCpR10. **(4)** *snr10.3* strain after spontaneous loss of YCpR10. The strain shown in sector 3 was grown for 24 h without selection in YEPD liquid medium and then plated on YEPD solid medium followed by replica plating to medium lacking uracil. 23% of colonies tested had lost the plasmid *URA3* marker, and all of these had returned to an *snr10.3* phenotype. Identical results were obtained using strains transformed to *snr10.1* or *.2*.

quid media at 30°C, $snr10^-$ strains have a doubling time 25% greater than otherwise isogenic *SNR10* strains; this difference increases to 47% at 25°C but does not appear to be further increased at lower temperatures. Strains lacking snR10 are also hypersensitive to elevated osmotic strengths. Of the solutes tested, the effects are most marked in 1 M KCl or 20% glycerol. The effects of these solutes and reduced temperature do not appear to be additive.

The relative growth of $snr10^-$ and *SNR10* strains on solid media is similar in comparisons on minimal versus complex media (YEPD), with fermentable versus non-fermentable carbon sources and at a pH range of 3 – 8. Diploids homozygous for *snr10.1* show similar growth properties to *snr10.1* haploid strains, but sporulation, spore germination and meiotic recombination appear unaffected (*snr10.2* and *snr10.3* have not yet been tested).

Microscopic examination of exponentially growing cultures reveals that, regardless of temperature, the increased doubling times of $snr10^-$ strains exactly correlates with an increase in the proportion of unbudded cells. It is thus likely that $snr10^-$ strains are delayed in leaving G1 (see Pringle and Hartwell, 1981). This behavior is consistent with a general metabolic defect, and inconsistent, for example, with a defect in DNA synthesis. In temperature shift experiments the growth rate of $snr10^-$ strains relative to isogenic *SNR10* strains responds promptly to either a rise or fall in temperature; that is the growth rate characteristic of a particular temperature is established well within a generation of the shift (data not shown).

As a first step in identifying the biochemical defect which underlies the observed growth phenotype, we measured the uptake of radioactive precursors into TCA-precipitable material in order to assess whether $snr10^-$ strains are impaired in net RNA or protein synthesis. During continuous growth at 22°C, *snr10.2* strains have a ratio of RNA synthesis to protein synthesis 23% lower than otherwise isogenic *SNR10* strains; following a temperature shift from 33 to 22°C this difference increases to 38%. Strains carrying *snr10.1* and *snr10.3* have also been shown to be relatively impaired in incorporation into RNA at 22°C.

Complementation test

Complementation tests were performed to demonstrate that the altered growth properties of $snr10^-$ strains are actually due to the absence of snR10, and not to some polar or other effect due to the insertion of the *LEU2* gene. The plasmid YCpR10 was constructed by subcloning the 1.05-kb *Hind*III fragment carrying the *SNR10* gene (Figure 1) into the low copy number yeast plasmid YCp50 (*CEN4, ARS1, URA3*) (C.Mann, personal communication). This region, which contains only 210 nucleotides of flanking sequences to each side of *SNR10*, is unlikely to encode another functional gene and does not hybridise to any other small RNA species.

YCpR10 fully complements *snr10.1, .2* and *.3* for cold sensitivity and osmotic sensitivity (Figure 6). Strains carrying the complementary plasmid spontaneously lose the plasmid marker at the expected frequency of ~2% per generation (Fitzgerald-Hayes *et al.*, 1982) and return to an $snr10^-$ phenotype.

Double mutants have been constructed carrying *snr10.1* and *snr3::LEU2* (Tollervey *et al.*, 1983). The growth of these strains is indistinguishable from *snr10.1*; $SNR3^+$ strains. *SNR10* and *SNR3* are unlinked genetically.

Discussion

Relationship to other snRNAs

As discussed previously (Wise *et al.*, 1983), snR10 and other yeast snRNAs resemble mammalian snRNAs in size, metabolic stability, possession of a 5′ cap structure containing 2,2,7-trimethyl guanosine and nuclear localisation. Moreover, the group of yeast RNAs which we have been examining represents the only possible analogues to the U-snRNAs of metazoan cells (Wise *et al.*, 1983; Guthrie *et al.*, 1985). However, snR10 does not show convincing primary sequence homology to any of the U-snRNAs. Three explanations for this finding appear possible. (i) The yeast snRNA has so far diverged evolutionarily from its mammalian analogue as to be now unrecognizable at the level of nucleotide sequence. (ii) There is no mammalian analogue. (iii) The mammalian analogue has yet to be identified.

The hypothesis that snR10 has diverged from some mammalian snRNA beyond the point of recognition is inconsistent with phylogenic comparisons based on other RNA species (Chen *et al.*, 1984; Gray *et al.*, 1984; Hori *et al.*, 1985). In the absence of knowing the role of snR10 in *S. cerevisiae* (see below), it is of course difficult to assess the possibility that there is no functional equivalent in metazoans. However, in that a cross-hybridising species appears to be present in the fungus *A. nidulans* it is probable that snR10-like snRNAs are at least not unique to yeast. The third possibility, moreover, is not unreasonable in the light of recent evidence that metazoan cells contain snRNAs other than the canonical six species U1 – U6. In addition to U7 (*op cit.*) Reddy and Busch (personal communication) have recently identified species designated U8, U9 and U10. Other RNA species with properties appropriate for snRNAs but which do not appear to correspond to U1 – U6 have been reported from *Dictyostelium* (Kaneda *et al.*, 1983), *Drosophila* (Wooley *et al.*, 1982) and mouse (Kato and Harada, 1984). It therefore seems conceivable that a snRNA with homology to snR10 may yet be identified in mammalian cells.

Implications for snR10 function

In striking contrast to strains lacking snR3, which have growth properties indistinguishable from wild-type sister strains under the extensive set of conditions tested (Tollervey *et al.*, 1983), the data we have presented here demonstrate that *snr10*$^-$ strains are considerably impaired in growth. The goal now is to determine the underlying biochemical basis for the biological phenotypes observed. The chief difficulty is that, in principle, the primary biochemical defect might be in almost any aspect of cellular metabolism. It is, therefore, useful to attempt to determine, in general terms, the processes which might be altered.

Based on the microscopic examination of *snr10*$^-$ cells, we can tentatively conclude that strains lacking snR10 are delayed in G1, presumably at the physiological step termed 'start' which entails a commitment to cell division (for a review, see Pringle and Hartwell, 1981). While this finding could be consistent with defects in many different aspects of general metabolism, it is inconsistent with defects in, for example, DNA replication or cytokinesis. The growth comparisons on minimal versus complex media and fermentable versus non-fermentable carbon sources indicates that the defect does not lie in a peripheral anabolic pathway, glycolysis, oxidative phosphorylation, the TCA cycle or any of the myriad functions required for mitochondrial maintenance.

The most intriguing aspect of the phenotype to be accounted for is its conditional and incomplete nature; that is, growth impairment is leaky at best and only measurable at or below 30°C or at high osmotic pressure. Since the shut-off in snR10 synthesis (at least in *snr10.3*) is complete under all conditions, the phenotype must be a reflection of the nature of the biochemical defect in these strains.

As first described a number of years ago (Guthrie *et al.*, 1969), cold-sensitive mutants in bacteria are frequently defective in ribosome assembly (for a review, see Jaskunas *et al.*, 1974). One reason for considering ribosome synthesis as a possible site of biochemical lesion in strains lacking snR10 is our observation that these strains exhibit a decreased ratio of RNA: protein synthesis at low temperatures. Since the majority of cellular RNA is rRNA, it is likely that the apparent reduction in incorporation into RNA in *snr10*$^-$ strains is due to a reduced rate of net rRNA synthesis. In principle such a reduction might be an indirect consequence of a defect in another aspect of metabolism. Hartwell (1967) reported that temperature-sensitive mutations not directly affecting RNA metabolism can result in a decreased ratio of RNA:protein synthesis. However, it is also possible that the absence of snR10 results in specific alterations in the rate of rRNA synthesis and/or breakdown. In any event this should be regarded only as a starting hypothesis for the detailed biochemical and genetic analyses, which we are now in a unique position to undertake, into the *in vivo* function of an snRNA.

Materials and methods

Media and strains

YEPD medium contained 10 g yeast extract, 20 g Bacto peptone (Difco) and 20 g glucose per litre (with 2% agar for solid media). For testing growth on non-fermentable carbon sources, the glucose was replaced by 2% glycerol or 50 mM sodium acetate. Minimal medium contained 6.7 g yeast nitrogen base without amino acids (Difco) and 20 g glucose per litre (with 2% agar for solid media). For testing growth at different pHs this medium was adjusted to the desired pH by the addition of HCl or NaOH (for media at pH 5 or less, 3% agar was used). Temperature shift experiments were performed using liquid YEPD medium.

The haploid strain which was used for transformation to *snr10*$^-$ carried *MATa, leu2-3, leu2-112, ura3-52, gal4-Δ 357, gal7-2, CAN1*^{R} (B.Patterson, personal communication). The diploid strain used for transformation to *snr10.1* carried *MATa/MATα, leu2-3/leu2-3, leu2-112/leu2-112, his4/+; his3/+; CAN1*^{R}*/CAN1*^{R}.

RNA extraction

Cultures (100 ml) were grown in YEPD medium, to OD_{600} 0.8 – 1. The cells were pelleted and resuspended in 0.5 ml buffer containing 100 mM sodium chloride, 50 mM sodium acetate pH 5, 20 mM EDTA and 0.1% SDS to which was added 0.5 ml phenol saturated with water and 0.5 g of glass beads (0.45 – 0.50 mm, B.Braun Melsungen). The mixture was agitated vigorously at room temperature for 6 min and then a further 10 ml of buffer and 10 ml of phenol were added. The mixture was incubated at 65°C for 20 min with agitation, 10 ml of chloroform was added and the aqueous phase was recovered after centrifugation. Following two further extractions with phenol/chloroform (1:1) the RNA was precipitated from the aqueous phase by the addition of 2.5 volumes of ethanol.

To prepare snR10 for sequencing, RNA of the appropriate mobility was electro-eluted from a 6% acrylamide, 7 M urea gel onto NA45 membrane (Schleicher and Schüll). The membrane was prepared by soaking for 10 min in 10 mM EDTA, followed by 5 min in 0.5 M NaOH and was rinsed several times in water. RNA was eluted by heating twice for 15 min at 68°C in buffer containing 1.2 M sodium chloride, 10 mM Tris-HCl, pH 8 and 1 mM EDTA. Following extraction with phenol/chloroform (1:1) the buffer was diluted 20% with water and the RNA recovered by ethanol precipitation.

For 3′ end labelling this fraction was then labelled with [5′-^{32}P]pCp using T4 RNA ligase (England and Uhlenbeck, 1978). For 5′ end labelling, RNA cap structures were first removed by oxidation with sodium *m*-periodate and β-elimination with analine (Rose and Lodish, 1976); the fraction was then labelled with [γ-^{32}P]ATP using T4 polynucleotide kinase. In each case snR10 was subsequently purified on two-dimensional polyacrylamide gels as described by Wise *et al.* (1983) except that the RNA was eluted from the second dimension via NA45 as described above.

DNA and RNA sequence analysis

The DNA sequence was determined by di-deoxynucleotide sequencing (Sanger *et al.*, 1977) of fragments subcloned into M13 (Messing *et al.*, 1981). The RNA sequence was determined by partial enzymatic digestion of end-labelled snR10

(Donnis-Keller *et al.*, 1977; Donnis-Keller, 1980) using RNase U2, A and PhyM (P-L) and T1 (Worthington).

Hybridisations

Southern hybridisation and Northern hybridisation from acrylamide gels were carried out as described by Wise *et al.* (1983). Northern hybridisation from agarose/formaldehyde gels was carried out as described by Jensen *et al.* (1983). Fragments of DNA for use as probes were prepared by elution from agarose gels using NA45 membrane (Schleicher and Schüll). Radioactive probes were prepared using nick-translation kit N 5,000 (Amersham).

Incorporation into RNA and protein

Relative incorporation into RNA and protein was measured as described by Hartwell (1967) using otherwise isogenic *SNR10* or *snr10.2* strains also carrying *ade1-100* and *lys2-1*. Exponentially growing cultures were labelled simultaneously with [^{14}C-8]adenine and [^{3}H]lysine in YM5 medium (Hartwell, 1967) for 1.5 h at 22°C or immediately following a temperature shift from 33 to 22°C. Relative incorporation into RNA and protein was compared in the *snr10.2* and *SNR10* strains.

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