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Cloning of the *RNA2* gene of *Saccharomyces cerevisiae*

Melanie G.Lee, Richard A.Young[1,2] and Jean D.Beggs

Cancer Research Campaign, Eukaryotic Molecular Genetics Research Group, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, UK and [1]Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305, USA
[2]Present address: Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02124, USA

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The *RNA2* gene of *Saccharomyces cerevisiae*, which has been implicated in splicing the transcripts of nuclear protein coding genes, has been cloned by complementation of the temperature-sensitive growth defect of an *rna2*-1 mutant strain. The cloned sequence also suppresses the accumulation of unspliced precursor transcripts of the actin gene in an *rna2*-1 mutant. The gene has been localised to a 3.2-kb DNA restriction fragment and the corresponding low abundance 2.8-kb transcript identified and the 5′ ends mapped. Evidence that this cloned sequence represents the *RNA2* gene includes homologous integration at the *rna2*-1 locus and disruption of the *RNA2* gene by insertional inactivation. The disrupted allele confers a recessive lethal phenotype, indicating an essential function for the *RNA2* gene product.

Key words: RNA2/splicing/yeast

Introduction

The *rna2*-1 mutation is a recessive conditional lesion in a gene which has been implicated in splicing the transcripts of nuclear protein coding genes in *Saccharomyces cerevisiae* (Teem and Rosbash, 1983). The *rna2* mutant, ts368, was among 400 temperature-sensitive (ts) mutants isolated by Hartwell (1967). Of these, 23 mutants showed rapid cessation of net RNA accumulation following a shift from 23°C to 36°C and subsequently were grouped into 10 complementation groups (*rna2* to *rna11*).

Shulman and Warner (1978) observed that precursor rRNA was synthesised but not efficiently processed in these mutants at the restrictive temperature, and Gorenstein and Warner (1976) showed that in the *rna* mutants the synthesis of ribosomal proteins co-ordinately decays to ~5-10% of wild-type levels during 1 h under non-permissive conditions. This decrease in protein synthesis was due to a reduction in the level of translatable ribosomal protein mRNA while the level of non-ribosomal protein mRNAs was not significantly affected (Hereford and Rosbash, 1977; Warner and Gorenstein, 1977). The kinetics of the decline in ribosomal protein mRNA synthesis and rRNA maturation in the mutants suggest that the defect in pre-rRNA processing may be a consequence of the depletion in ribosomal proteins (Gorenstein and Warner, 1976).

Molecular analyses by Rosbash *et al.* (1981) and Fried *et al.* (1981) revealed that at the restrictive temperature *rna2* strains accumulate high mol. wt. precursor transcripts of intron-containing ribosomal protein genes although the total amount of all transcripts of these genes declines. Measurements of rates of transcription of ribosomal protein genes indicated that the depletion of these transcripts at the non-permissive temperature is a consequence of the rapid turnover of the unprocessed molecules and that their rate of synthesis is relatively unaffected (Bromley *et al.*, 1982; Kim and Warner, 1983). More recently it has been shown that the effect of the *rna2* mutation is not solely upon the ribosomal protein genes. The transcripts of the intron-containing actin and *MAT*a1 genes are similarly affected (Teem *et al.*, 1983; Miller, 1984). Also, using a hybrid construct Teem and Rosbash (1983) have shown that the mere presence of a yeast intron is sufficient to render a message sensitive to the *rna2* mutation. Although the transcripts of higher eukaryotic genes are not generally spliced in *S. cerevisiae*, Watts *et al.* (1983) showed that transcripts of the *Drosophila melanogaster* alcohol dehydrogenase gene are processed in an *RNA2*-dependent reaction. Perhaps a clue to the understanding of the above findings is the demonstration that certain post-precursor intermediate structures in the splicing reaction are not observed in *rna2* strains at 36°C (Pikielny *et al.*, 1983).

We have isolated the *RNA2* gene by complementation of the temperature-sensitive growth defect of an *rna* mutant strain. The presence of the cloned gene in the mutant strain also suppresses the accumulation of unspliced actin precursor transcripts. The *RNA2* gene is unique in the yeast genome. By replacing the genomic copy with an allele bearing an *in vitro* generated mutation we have shown the *RNA2* gene to be essential.

Results

Cloning of the RNA2 gene

The *RNA2* gene was isolated from a yeast genomic DNA library constructed by Carlson and Botstein (1982). This library is composed of fragments from a *Sau*3A partial digest of DBY939 (S288C*suc2-215am*) DNA cloned in the yeast plasmid vector YEp24 (Botstein *et al.*, 1979), which carries the *URA3* gene. *S. cerevisiae* strain RY26 (Table I), which carries the *rna2*-1 mutation and is *ura3*$^-$, was transformed with the plasmid library DNA and Ura$^+$ transformants were selected at the non-permissive temperature (36°C). A single transformant was obtained from which the plasmid pY2000 was extracted. pY2000 DNA reproducibly confers the ability to grow at 36°C (non-ts) on transformants of four strains (Figure 1) carrying the *rna2*-1 allele. Plasmid pY2000 contains an insert of 7.7 kb of *S. cerevisiae* DNA. This insert was subcloned in various yeast vectors and the gene which complements the *rna2*-1 mutation was localised to the 3.2-kb *Eco*RI-*Bam*HI region present in pY2076 and pY2016 (Figure 1).

To investigate whether the cloned gene represents a unique sequence in the *S. cerevisiae* genome a gel transfer hybridisation experiment was done. When the 3.2-kb *Eco*RI-*Bam*HI DNA fragment is radioactively labelled and hybridised to

Table I. The genotypes and origins of strains of *S. cerevisiae* used in this work

Strain	Genotype	Origin
ts368	*a rna2-1 ura1 ade1 ade2 his7 lys2 tyr1 gal1*	Hartwell *et al.*, 1970
SR14-2	*a ura3-1 ura3-2 ade2-1 can*R *ino gal2*	D.Botstein, M.I.T.
RY26	*α rna2-1 ura3-1 ura3-2 ade1 ade2 tyr1 his7 can*R *GAL*	ts368 was twice outcrossed with SR14-2. RY26 is a ts$^-$ progeny of the second cross
LL20	*α leu2-3 leu2-112 his3-11 his3-15 can1*	Gerald Fink, Cornell University
20243	*α rna2-1 leu2-3 leu2-112 his3-11 his3-15*	One of the ts$^-$ progeny of ts368 crossed with LL20; Viesturs Simanis, Imperial College
RL92	*a rna2-1 leu2-3 leu2-112 ura3-52*	Robert Last, Carnegie-Mellon University
DBY747	*a ura3-52 leu2-3 leu2-112 his3-1 trp1-289*	David Botstein, M.I.T.
MGL1	*a rna2-1 leu2-3 leu2-112 ura3 trp1-289 ade1/2 his*	Derived in this work by crossing RY26 with DBY747
SPJO.9	*α leu2-3 leu2-112 ura3-52 his3-1 lys2*	Stephen Jackson, Imperial College

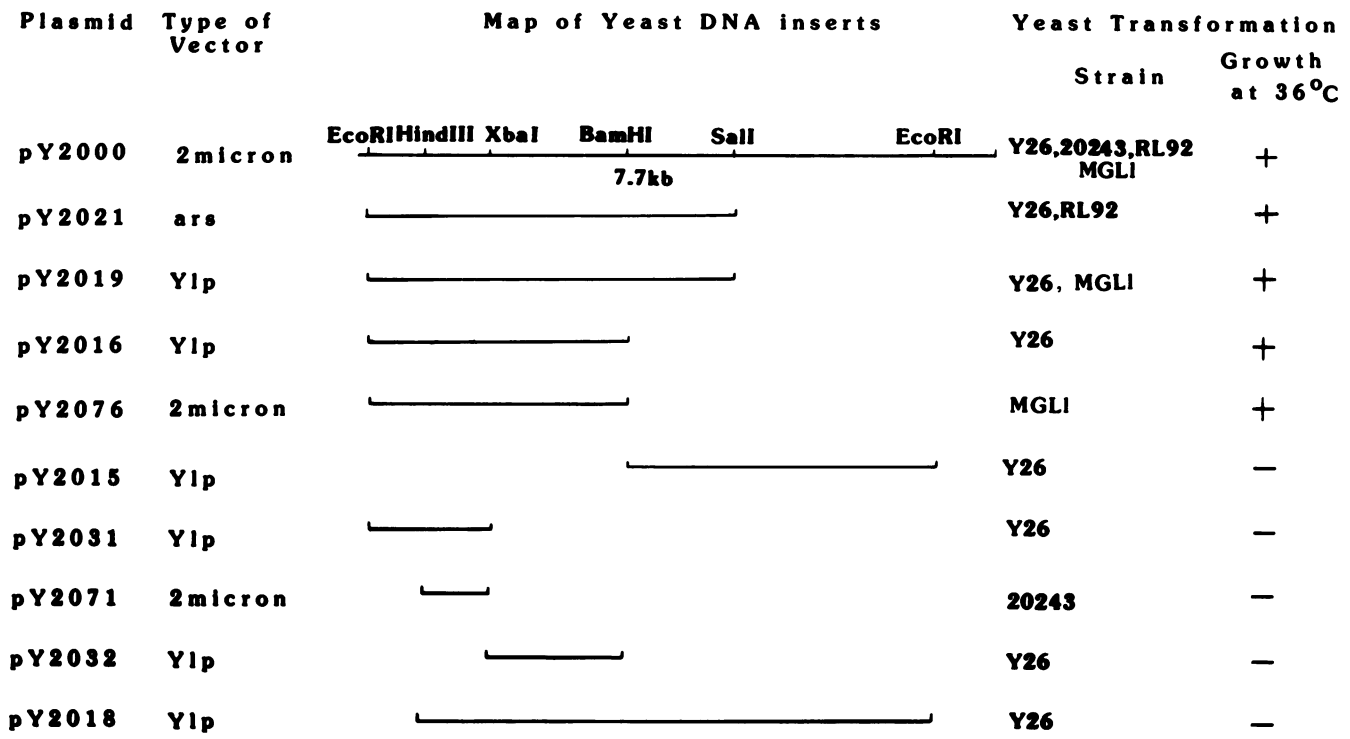

Fig. 1. Restriction map of the *RNA2* locus and complementation results for various subclones. The right hand columns show the yeast strains in which complementation of the *rna2-1* mutation was tested; + indicates complementation by direct selection of transformants at 36°C, − indicates inability to produce transformants by direct selection at 36°C. Only those restriction sites important for construction of clones used in this work are shown. The 2 μm vectors used to construct pY2000 and pY2076 were YEp24 (Botstein *et al.*, 1979) and pJDB207 (Beggs, 1981), respectively.

Southern blots (Southern, 1975) of genomic *Eco*RI, *Hind*III or *Sal*I digestion fragments (Figure 2), a single, strongly hybridising band is seen for the *Eco*RI or *Sal*I digested DNA, and two strong signals for *Hind*III digested DNA, as expected for a unique sequence. Hybridisation under conditions of reduced stringency (25% formamide at 42°C; not presented) shows the presence of multiple fragments having weak homology with the cloned sequence, suggesting that part of this sequence may be repeated elsewhere in the genome, although the significance of this is not yet clear.

Complementation of the RNA splicing defect

At the non-permissive temperature, cells carrying the *rna2-1* mutation exhibit a dramatic decrease in the levels of mature mRNA and a corresponding increase in the amounts of unspliced precursor transcripts of nuclear protein-coding genes containing introns (Rosbash *et al.*, 1981; Teem and Rosbash, 1983; Teem *et al.*, 1983; Larkin and Woolford, 1983). Figure 3a shows a comparison of actin gene transcripts from LL20 (*RNA2*$^+$) and 20243 (*rna2-1*) and the effect of incubation at 36°C for 1 h. Actin precursor transcripts (1700 nucleotides) accumulate in 20243 at 36°C (lane 4), and the level of spliced transcripts (1400 nucleotides) is considerably reduced. One or more actin-specific species with an electrophoretic mobility between that of full-length precursor and that of mature transcript are observed in LL20 at both temperatures. These are no longer seen in 20243 after 1 h at 36°C. The nature of these RNA species is not known but they may represent splicing intermediates such as those found during the splicing of adenovirus 2 transcripts in HeLa cell extracts (Grabowski *et al.*, 1984). The 830 nucleotide RNA corresponds to the *protein2* (*YP2*) transcript, the sequence of which is present on the DNA probe used here (Gallwitz and Sures, 1980). This transcript is not sensitive to the *rna2-1* mutation and acts as a useful reference. Following incubation at 36°C, RY26 (*rna2-1*) cells transformed with the cloned sequence on an integrating vector (pY2019), on a 2 μm DNA based vector (pY2000), or on an *ars* vector (pY2021) show

(Figure 2b) neither the accumulation of actin precursor RNA nor the depletion of mature actin transcripts observed with untransformed RY26 cells or RY26 cells carrying plasmid vector sequences only (not shown). Thus the cloned gene complements the effect of the *rna2*-1 mutation at the level of RNA splicing as well as reversing the temperature-sensitive growth defect.

Genetic relationship of the cloned sequence to the rna2-1 locus

Plasmid DNAs such as pY2019 which cannot replicate autonomously in yeast cells can be stably maintained in yeast transformants through homologous integration into the yeast genome (Hinnen *et al.*, 1978). Orr-Weaver *et al.* (1981) showed that linear DNAs preferentially integrate at the chromosomal locus which has homology with the ends of the linear molecules. Genetic mapping of a homologously integrated gene can thus provide evidence that this gene corresponds to a particular genetic locus. To promote integration at the genomic locus corresponding to the *rna2*-complementing sequence, pY2019 DNA was linearised by digestion with *Hind*III. A pY2019 transformant (phenotypically Ura$^+$ and non-ts) of RY26 was crossed with the *ura3*$^-$ *RNA2*$^+$ strain DBY747. Diploids were sporulated and six tetrads were dissected and analysed for segregation of the Ura$^+$ and ts phenotypes. Ura$^+$ segregated 2$^+$:2$^-$ and since the *URA3* gene was located on the pY2019 DNA this demonstrates that the integration event occurred at a nuclear chromosomal locus. None of the haploid progeny were temperature sensitive, indicating linkage of the pY2019 sequence (and the ts-complementing gene) to the *rna2*-1 locus. Precise localisation of the integration event at *rna2*-1 would require a more extensive analysis; however, the gene disruption experiment described below is more conclusive.

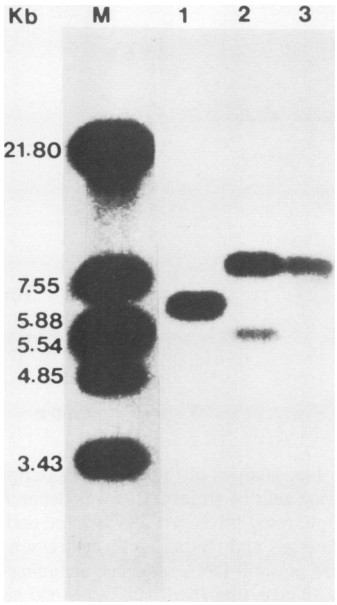

Fig. 2. Southern blot analysis of total genomic DNA from strain SPJO.9 digested to completion with either *Eco*RI **(lane 1)**, *Hind*III **(lane 2)** or *Sal*I **(lane 3)**. The genomic DNAs and DNA size markers **(lane M)** were electrophoresed through 0.8% agarose and blotted onto Gene Screen Plus (New England Nuclear). The filter was hybridised with a uniformly ^{32}P-labelled *Eco*RI-*Bam*HI DNA fragment of the *rna2*-1 gene which was gel purified from an *rna2*-lambda clone. (The lambda DNA marker track was separately hybridised with a lambda probe.) Hybridisation was performed by the recommended protocol of the Gene Screen Plus manufacturers (New England Nuclear) for hybridisation in formamide at 42°C.

Disruption of the RNA2 gene

Various procedures have been described by which a locus may be disrupted or replaced by a homologous sequence which has been cloned and altered *in vitro*. This approach has been used to replace the wild-type *HIS3* gene by a deleted derivative (Scherer and Davis, 1979), to demonstrate that a cloned gene corresponds to a particular locus (Neff *et al.*, 1983) and to determine whether a cloned sequence has an essential function *in vivo* (Shortle *et al.*, 1982; Neff *et al.*, 1983; Tollervey *et al.*, 1983). The cloned *rna2*-1 complementing sequence was disrupted *in vitro* by the insertion of the *URA3* gene at the *Hind*III site to produce the construct *RNA2*::*Hind*III (Figure 4a). This construct was used to replace the homologous chromosomal sequence by the method of Rothstein (1983) to provide conclusive evidence that the cloned sequence corresponds to the *RNA2* locus and to determine whether *RNA2* function is essential for cell

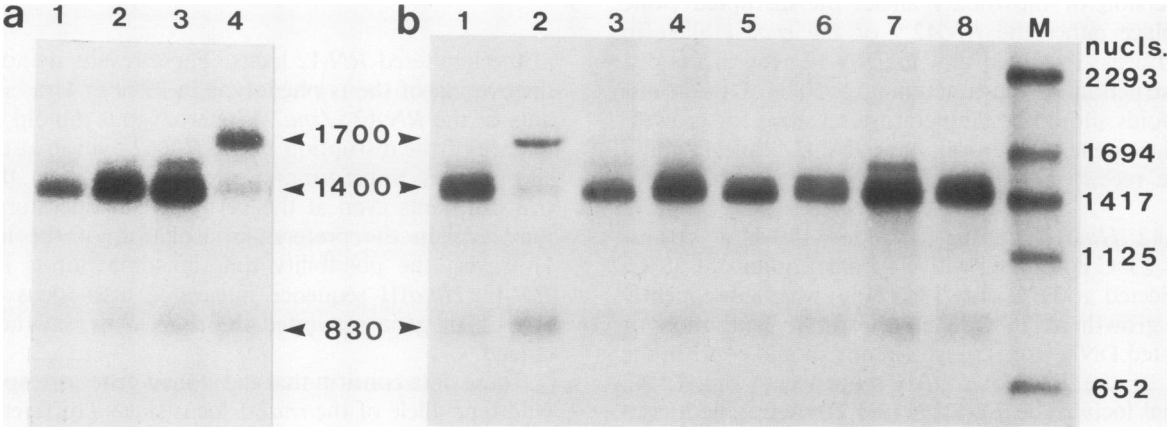

Fig. 3. Northern blot analysis of actin transcripts in *RNA2*$^+$ and *rna2*-1 strains, and *rna2*-1 strains transformed with the cloned *RNA2* gene. RNA or DNA size markers were denatured with glyoxal, electrophoresed through 1.5% agarose and transferred to Gene Screen (see Materials and methods). Filters were hybridised with uniformly ^{32}P-labelled pYA208 DNA **(a)** or the gel purified *Pst*I-*Eco*RI fragment from pYA208, containing the actin sequence but free from pBR322 sequence **(b)**. **(a)** Poly(A)$^+$ RNA from LL20 (*RNA2*$^+$; **lanes 1** and **2)** or 20243 (*rna2*-1; **lanes 3** and **4)** grown at 23°C **(lanes 1** and **3)** or shifted to 36°C for 1 h **(lanes 2** and **4)**. **(b)** 18 μg of total RNA from RY26 **(lanes 1** and **2)**, or RY26 transformed with pY2019 **(lanes 3** and **4)**, pY2000 **(lanes 5** and **6)** or pY2021 **(lanes 7** and **8)** grown at 23°C **(lanes 1, 3, 5** and **7)** or shifted to 36°C for 1 h **(lanes 2, 4, 6** and **8)**. **Lane M** contains DNA size markers with sizes indicated in nucleotides (nucls.).

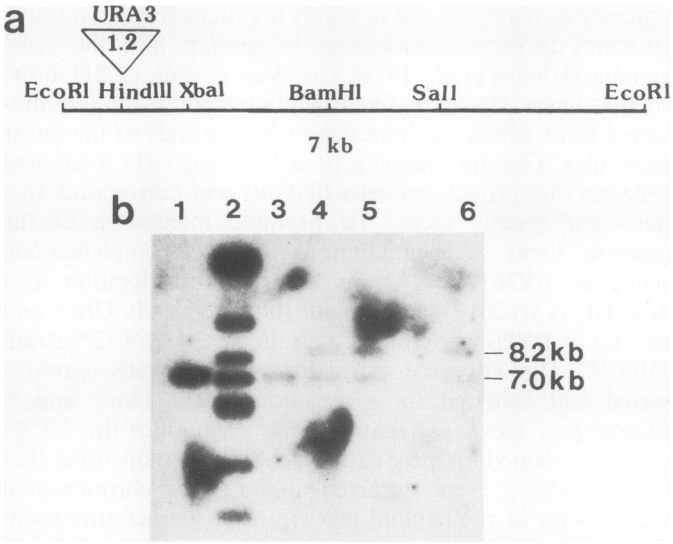

Fig. 4. Southern blot analysis of DNA from cells transformed with the *RNA2*::*Hind*III recombinant sequence. **(a)** Schematic representation of the *RNA2* locus. Only those restriction endonuclease cleavage sites important for the formation of recombinant clones used in this work are indicated. In the *RNA2*::*Hind*III construct a 1.2-kb *Hind*III DNA fragment containing the *URA3* gene of *S. cerevisiae* was cloned into the *Hind*III site of the *RNA2* sequence. Replacement of the *RNA2* locus by the *RNA2*::*Hind*III recombinant sequence should increase the size of the *Eco*RI digestion fragment at that locus from 7.0 kb to 8.2 kb. **(b)** DNA was digested with *Eco*RI and electrophoresed through 0.8% agarose and transferred to Gene Screen Plus. The filter-bound DNA was hybridised with a uniformly ^{32}P-labelled lambda DNA clone containing an *Eco*RI restriction fragment with the *rna2*-1 gene sequence. Autoradiography was carried out as described in Materials and methods. **Lane 1**, pY2000 DNA; **2**, lambda DNA digested with *Eco*RI and *Hind*III; **3**, DNA from untransformed MGL1 x SPJO.9; **4, 5** and **6**, DNA from cells transformed with *RNA2*::*Hind*III: **lane 4**, X21 (ts); **lane 5**, X22 (ts); **lane 6**, X212 (non-ts).

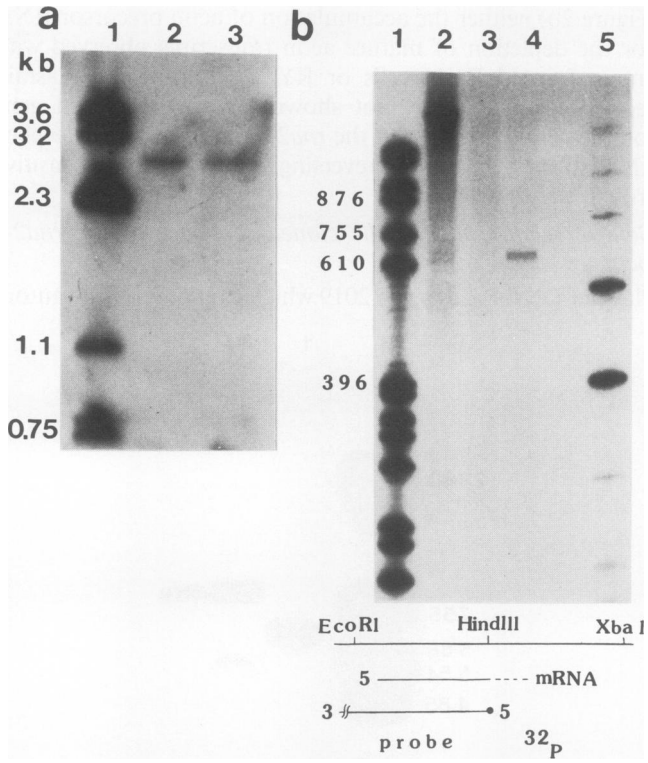

Fig. 5. (a) Northern blot analysis of the *RNA2* transcript. RNA from logarithmically growing cells of strain 20243 was denatured by incubation with glyoxal, electrophoresed through 1.5% agarose and transferred to Gene Screen (see Materials and methods). Hybridisation was to a uniformly ^{32}P-labelled plasmid DNA sequence including the *Eco*RI-*Pvu*II fragment of the *RNA2* gene (the *Pvu*II site lies ~600 nucleotides to the right of the *Xba*I site shown in Figure 1). **Lane 1**, pBR322 DNA size markers; **2**, RNA from 20243 grown at 23°C, or **3**, shifted to 36°C for 1 h. **(b)** S1 nuclease assay to map the 5′ ends of *RNA2* gene transcripts. The diagram shows the origin of the DNA probe which was 5′ ^{32}P-labelled at the *Hind*III site within the *RNA2* gene, and extended to the *Eco*RI site flanking the *RNA2* gene. RNA (from logarithmically growing cells of strain LL20) and DNA probe were annealed at 46°C and digested with 200 units of S1 nuclease/ml at 20°C for 1 h. The protected fragments were denatured and detected by electrophoresis through a 5% polyacrylamide gel containing 7 M urea, followed by autoradiography. **Lanes 1** and **5**, DNA size markers; **2**, undigested probe; **3**, control digest without yeast RNA; **4**, RNA from LL20.

viability. The *Eco*RI-*Sal*I DNA fragment containing the *RNA2*::*Hind*III construct was gel purified and used to transform a diploid strain (*MATα/MAT*a, *RNA2*$^+$/*rna2*-1, *ura3*/*ura3*) which was constructed by mating SPJO.9 and MGL1 (Table I). This *RNA2*$^+$/*rna2*-1 heterozygous diploid grows normally at 36°C due to the dominance of the Rna2$^+$ phenotype. If the cloned gene does indeed correspond to the wild-type version of the *rna2*-1 allele, the disrupted clone should displace either the *RNA2*$^+$ or the *rna2*-1 allele in transformed cells. Provided that the *RNA2* gene in the disrupted construction is non-functional, ~50% of the transformed diploids should be temperature sensitive for growth, as the displacement of the dominant *RNA2*$^+$ allele should uncover the recessive temperature-sensitive growth phenotype.

The *RNA2*::*Hind*III construct yielded 189 Ura$^+$ transformants at 23°C compared with 88 transformants at 36°C. Of those selected at 23°C only 134 (71%) were subsequently capable of growth at 36°C. Gel transfer hybridisation of *Eco*RI digested DNA from transformants should confirm the localisation of the *RNA2*::*Hind*III sequence at the *RNA2* chromosomal locus. The 7.0-kb *Eco*RI DNA fragment containing the *RNA2* gene should increase in size to 8.2 kb when it contains the *URA3*-disrupted sequence (Figure 4a). In Figure 4b the *Eco*RI DNA fragments homologous to an *RNA2*-specific radiolabelled probe are shown for two temperature-sensitive (ts) Ura$^+$ transformants, X21 and X22 and a non-ts Ura$^+$ transformant, X212. In each case a band of 8.2 kb is observed in addition to the 7.0-kb sequence present at the unaltered *RNA2* locus. These results demonstrate the uncovering of the ts phenotype in 29% of Ura$^+$ transformants of the *RNA2*$^+$/*rna2*-1 heterozygous diploid as a consequence of the disruption of the *RNA2*$^+$ allele at the *Hind*III site. The ts transformants grow more slowly than non-ts transformants even at the permissive temperature, and this may explain the preferential isolation of the latter class. However, the possibility that in some non-ts isolates the *RNA2*::*Hind*III sequence integrated at a locus other than *RNA2*, or gene converted the *ura3* locus, has not been excluded.

These data confirm that the cloned gene corresponds to the wild-type allele of the *rna2*-1 locus since a different sequence with the ability to suppress the ts phenotype would not disrupt the dominant *RNA2*$^+$ allele of the heterozygous diploid strain. In addition, these results verify that the *Hind*III site at which the *URA3* gene was inserted lies in a sequence essential for functional expression of *RNA2*.

A Ura$^+$ ts transformant (X22), a Ura$^+$ non-ts transformant (X212) and the untransformed diploid MGL1 x SPJO.9

were sporulated and the spores were dispersed and regenerated on complete medium at 23°C. Approximately 100 haploid progeny of each diploid were tested for the ability to grow at 36°C and for the Ura$^+$ phenotype which represents the *RNA2*::*Hind*III allele. All haploid progeny of the Ura$^+$ transformants X22 and X212 were Ura$^-$, and ts or non-ts respectively. The failure to isolate any Ura$^+$ haploids indicates that the *RNA2*::*Hind*III disrupted allele represents a recessive lethal mutation, and that the *RNA2* gene product is essential for spore germination and/or cell viability.

Analysis of RNA2 transcripts

Gel transfer of RNA and hybridisation with a radiolabelled probe homologous to the *Eco*RI-*Pvu*II region (2.2 kb) of the *RNA2* gene identifies a transcript 2.8 kb long (Figure 5a). The same result was obtained with RNA from an *rna2*-1 strain grown at 23°C (lane 2) or shifted to 36°C for 1 h (lane 3) and also from *RNA2*$^+$ strains (not shown), indicating that the mutant phenotype is not mediated by a substantial change in the size or abundance of the gene transcript. The *RNA2* transcripts are in relatively low abundance, being present at ~1% of the level of actin and ribosomal protein gene transcripts, or <0.01% of poly(A)$^+$ RNA (data not shown). Using single-stranded probes produced from the *RNA2* sequence cloned in M13 bacteriophage vectors, the direction of transcription was shown to be from left to right on the map as represented in Figure 1.

S1 nuclease mapping confirmed this assignment of the coding strand and located the 5′ ends of the transcripts between the *Eco*RI and *Hind*III sites. Figure 5b shows the results of S1 nuclease digestion using a DNA probe 5′ end-labelled at the *Hind*III site and extending to the *Eco*RI site. Approximately 635 ± 10 nucleotides of the probe were protected against S1 nuclease digestion, localising the 5′ ends of the transcripts ~180 nucleotides to the right of the *Eco*RI site. If there is a short intervening sequence very close to the 5′ end of the gene these S1 mapping data could represent the position of a splice junction rather than a 5′ terminus. However, preliminary DNA sequence data indicate the absence of intron consensus sequences in this region (Watts, Smith and Beggs, unpublished results). The 3.2-kb *Eco*RI-*Bam*HI sequence is therefore long enough to encode the *RNA2* transcript.

Discussion

A gene from *S. cerevisiae* has been cloned which complements the temperature-sensitive growth and RNA splicing defects resulting from the *rna2*-1 mutation. The gene has been characterised by restriction mapping and subcloning and a 2.8-kb transcript identified. That this cloned DNA sequence corresponds to the *RNA2* gene rather than to an extragenic suppressor was shown by tetrad analysis of the homologously integrated sequence and by inactivation of *RNA2* function upon disruption of the gene.

The inability of *rna2* mutant strains to grow at 36°C suggests that the *RNA2* gene product is essential for cell growth and even for cell viability; within 5 h of shifting strain RY26 to 36°C the viable cell count drops to 10% (Rahman and Lee, unpublished results). However, studies of the *rna2* mutant could produce misleading results if the product of the mutant allele has a novel activity at 36°C. In the gene disruption experiment described here the failure to isolate any Ura$^+$ haploid progeny from the *RNA2*::*Hind*III diploid transformants confirms that the *RNA2* gene product is essential for spore germination and/or cell viability. The lethal effect of the *rna* mutations is likely to be a consequence of the RNA processing defect which results in the inability to produce some essential proteins including actin (Teem *et al.*, 1983) and many ribosomal proteins (Rosbash *et al.*, 1981; Fried *et al.*, 1981).

The nature and function of the *RNA2* gene product remain to be determined. The RNA splicing defect observed in *rna2*-1 mutants at the non-permissive temperature may not be the primary defect but could occur as a consequence of a block at another step in RNA maturation. By analogy, a *Neurospora* nuclear mutation *cyt*-4 causes the accumulation of unspliced mitochondrial large rRNA with short 3′ end extensions. It is proposed that the aberrant 3′ ends result in incorrect folding of the transcripts rendering them unable to be spliced (Garriga *et al.*, 1984).

We have cloned the *RNA2* gene with a view to investigating its function and thereby improving our understanding of RNA processing in yeast. We are investigating the effect of the cloned gene on the transcripts of intron-containing genes and putative splicing intermediates under various controlled conditions. Preliminary DNA sequencing analysis of the *RNA2* gene has shown the presence of a long open reading frame corresponding to the transcribed region (Watts, Smith and Beggs, unpublished results). We therefore anticipate that the *RNA2* gene encodes a protein and we have initiated immunological studies to identify and characterise the protein and investigate its interaction with other macromolecules *in vivo* and the nature of its role in RNA processing.

Materials and methods

Enzymes

Restriction enzymes were purchased from New England Biolabs or Boehringer Company Ltd. and used according to the manufacturers recommendations. DNA ligase was a gift from J.Wolfe and D.Smith (Imperial College). S1 nuclease was obtained from Sigma, and calf intestinal phosphatase and T4 polynucleotide kinase were from Boehringer Company Ltd. Radiochemicals, *Escherichia coli* DNA polymerase I holoenzyme and Klenow fragment were purchased from Amersham International.

Strains and DNA constructs

E. coli strains HB101 (Boyer and Roulland-Dussoix, 1969) or DB6656 (Botstein *et al.*, 1979) were used for the cloning and amplification of plasmid DNAs. *E. coli* strain NM514 (*hfl*$^-$ *hsd*R$^-$; Murray, 1984) was used for the propagation of lambda clones. The *rna2*-1 allele from strain 20243, cloned as a 7.0-kb *Eco*RI DNA fragment in the lambda vector NM1150 (Murray, 1984) was used as an RNA2-specific probe where indicated. The *S. cerevisiae* (strain DBY939) *Sau*3A library in YEp24 (Carlson and Botstein, 1982) and the plasmid vectors YEp24, YRp17 and YIp5 were provided by David Botstein. pYA208 containing the yeast actin gene was from D.Gallwitz (Gallwitz and Sures, 1980). For the *RNA2* gene disruption construct the plasmid YEp24 (Botstein *et al.*, 1979) was the source of the 1.2-kb *Hind*III DNA fragment containing the *URA3* gene. *S. cerevisiae* strains and their origins are listed in Table I.

Transformation and microbiological procedures

The compositions of media for growth of *S. cerevisiae* were as described by Beggs (1978) and Sherman *et al.* (1983). The yeast transformation procedure was essentially as described by Beggs (1978) except that the length of the incubation of spheroplasts with polyethylene glycol was kept to a minimum and 10 mM $CaCl_2$ was present in the final sorbitol wash of spheroplasts. Sonicated salmon sperm DNA (100 μg/ml) was included in the gene disruption experiment as described by Rothstein (1983). Temperature-sensitive strains were maintained without shaking, at 23°C throughout the procedure, and after plating were incubated at 23°C for 12−24 h before transferring to 36°C. Yeast tetrad analysis and other standard yeast genetic techniques were performed as described by Mortimer and Hawthorne (1973).

Nucleic acid preparations

Small-scale yeast DNA extractions were performed as described by Beggs (1978) or Davis *et al.* (1980). Yeast genomic DNA was prepared on a large

scale as described by Cryer *et al.* (1975). Recombinant phage and plasmids were constructed essentially as described by Maniatis *et al.* (1982) and plasmids in bacterial cells were analysed using the rapid extraction method of Birnboim and Doly (1979). DNA fragments were purified from agarose by binding to ground glass in the presence of sodium iodide (Vogelstein and Gillespie, 1979). Yeast RNA was extracted essentially as described by Hopper *et al.* (1978) from cultures grown at 23°C or shifted to 36°C by the addition of an equal volume of medium at 49°C 1 h prior to extraction. Poly(A)$^+$ and poly(A)$^-$ species were separated by one passage over oligo(dT)-cellulose (Aviv and Leder, 1972).

S1 nuclease mapping

S1 nuclease digestions were carried out by the method of Berk and Sharp (1977) as modified by Weaver and Weissmann (1979). DNA probes were 5′ end-labelled according to Maniatis *et al.* (1982).

Filter hybridisations

Plaque hybridisations to identify a lambda clone containing the *rna*2-1 sequence were carried out as described by Maniatis *et al.* (1982) using pY2000 DNA as a probe. For gel transfer hybridisation, DNA samples were electrophoresed through agarose and transferred to Gene Screen Plus (New England Nuclear) and hybridised according to the manufacturer's recommendations.

RNA [total or poly(A)$^+$] was denatured by incubation with glyoxal (McMaster and Carmichael, 1977) followed by electrophoresis in 1.5% or 1.7% agarose in 10 mM sodium phosphate pH 7.0. The RNA was transferred by blotting with 1 x SSC (0.15 M sodium chloride, 0.015 M trisodium citrate) onto Gene Screen (New England Nuclear) and hybridised and washed according to the manufacturers recommendations (without dextran sulphate). DNA probes were uniformly labelled by nick-translation (Rigby *et al.*, 1977) or end-labelled according to Maniatis *et al.* (1982). Single-stranded probes were prepared by primer extension on M13 clone templates followed by digestion with an appropriate restriction endonuclease and gel purification of the newly synthesised ^{32}P-labelled strand under denaturing conditions (O'Hare *et al.*, 1983). Autoradiography was carried out at −70°C using Fuji RX film and Mach 2 intensifying screens.

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References

Aviv,J. and Leder,P. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 1408-1412.
Beggs,J.D. (1978) *Nature*, **275**, 104-109.
Beggs,J.D. (1981) in Von Wettstein,D., Friis,J., Kielland-Brand,M. and Stenderup,A. (eds.), *Molecular Genetics in Yeast*, Alfred Benzon Symposium, Vol. **16**, Munksgaard, Copenhagen, pp. 383-395.
Berk,A.J. and Sharp,P.A. (1977) *Cell*, **12**, 721-732.
Birnboim,H.C. and Doly,J. (1979) *Nucleic Acids Res.*, **7**, 1513-1523.
Botstein,D., Falco,S.C., Stewart,S.E., Brennan,M., Scherer,S., Stinchcomb, D.T., Struhl,K. and Davis,R.W. (1979) *Gene*, **8**, 17-24.
Boyer,H.W. and Roulland-Dussoix,D. (1969) *J. Mol. Biol.*, **41**, 459-472.
Bromley,S., Hereford,L. and Rosbash,M. (1982) *Mol. Cell. Biol.*, **2**, 1205-1211.
Carlson,M. and Botstein,D. (1982) *Cell*, **28**, 145-154.
Cryer,D., Eccleshall,R. and Marmur,J. (1975) *Methods Cell Biol.*, **12**, 39-44.
Davis,R.W., Thomas,M., Cameron,J., St.John,T.P., Scherer,S. and Padgett,R.A. (1980) *Methods Enzymol.*, **65**, 404-411.
Fried,H.M., Pearson,N.J., Kim,C.H. and Warner,J.R. (1981) *J. Biol. Chem.*, **256**, 10176-10183.
Gallwitz,D. and Sures,I. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 2546-2550.
Garriga,G., Bertrand,H. and Lambowitz,A.M. (1984) *Cell*, **36**, 623-634.
Gorenstein,C.G. and Warner,J.R. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 1547-1551.
Grabowski,P.J., Padgett,R.A. and Sharp,P.A. (1984) *Cell*, **37**, 415-427.
Hartwell,L.H. (1967) *J. Bacteriol.*, **93**, 1662-1670.
Hartwell,L.H., McLaughlin,C.S. and Warner,J.R. (1970) *Mol. Gen. Genet.*, **109**, 42-56.
Hereford,L.M. and Rosbash,M. (1977) *Mol. Gen. Genet.*, **10**, 463-467.
Hinnen,A., Hicks,J.B. and Fink,G.R. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1929-1933.
Hopper,A.K., Banks,F. and Evangelidis,V. (1978) *Cell*, **14**, 211-219.
Kim,C.H. and Warner,J.R. (1983) *Mol. Cell. Biol.*, **3**, 457-465.
Larkin,J.C. and Woolford,J.L. (1983) *Nucleic Acids Res.*, **11**, 403-420.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
McMaster,L.K. and Carmichael,G.G. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 4835-4838.
Miller,A.M. (1984) *EMBO J.*, **3**, 1061-1065.
Mortimer,R. and Hawthorne,D. (1973) *Genetics*, **74**, 33-54.
Murray,N.E. (1984) in Hendrix,R.W., Roberts,J.W., Stahl,F.W. and Weisberg,R.A. (eds.), *Lambda 2*, Cold Spring Harbor Laboratory Press, NY, pp. 395-432.
Neff,N.F., Thomas,J.H., Grisafi,P. and Botstein,D. (1983) *Cell*, **33**, 211-219.
O'Hare,K., Levis,R. and Rubin,G.M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 6917-6921.
Orr-Weaver,T.L., Szostak,J.W. and Rothstein,R.J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6354-6358.
Pikielny,C.W., Teem,J.L. and Rosbash,M. (1983) *Cell*, **34**, 395-403.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.
Rosbash,M., Harris,P.K.W., Woolford,J.L. and Teem,J.L. (1981) *Cell*, **24**, 679-686.
Rothstein,R.J. (1983) *Methods Enzymol.*, **101**, 202-211.
Scherer,S. and Davis,R.W. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4951-4955.
Sherman,F., Fink,G.R. and Hicks,J.B. (1983) *Methods in Yeast Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
Shortle,D., Haber,J.E. and Botstein,D. (1982) *Science (Wash.)*, **217**, 371-373.
Shulman,R.W. and Warner,J.R. (1978) *Mol. Gen. Genet.*, **161**, 221-223.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.
Teem,J.L. and Rosbash,M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 4403-4407.
Teem,J.L., Rodriguez,J.R., Tung,L. and Rosbash,M. (1983) *Mol. Gen. Genet.*, **192**, 101-103.
Tollervey,D., Wise,J.A. and Guthrie,C. (1983) *Cell*, **35**, 753-762.
Vogelstein,B. and Gillespie,D. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 615-619.
Warner,J.R. and Gorenstein,C. (1977) *Cell*, **11**, 201-212.
Watts,F.Z., Castle,C. and Beggs,J.D. (1983) *EMBO J.*, **2**, 2085-2091.
Weaver,R.F. and Weissmann,C. (1979) *Nucleic Acids Res.*, **7**, 1175-1193.

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