The Effect of Temperature-Sensitive RNA Mutants on the Transcription Products from Cloned Ribosomal Protein Genes of Yeast

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Summary

The levels of four ribosomal protein (rp) mRNAs in different mutant strains were determined by hybridization of radiolabeled cloned genes to RNA fractionated on CH$_2$O$_3$ paper. Two ribosomal protein genes (rp 51 and rp 52) controlled by the locus RNA2 have dramatically decreased mRNA levels after a shift-up to the nonpermissive temperature in a strain carrying the rna2 mutation (ts368). Two ribosomal protein genes not controlled by the RNA2 locus and several control nonribosomal protein genes are relatively unaffected by the temperature shift in this strain. Other genes in the vicinity of one of the rna2-sensitive ribosomal protein genes (the rp 51 gene) are insensitive to the rna2 gene product, suggesting that all ribosomal protein genes do not occur in clusters and that the RNA2 gene product does not affect a large region of chromatin. In ts368 at the nonpermissive temperature, the concentration of higher molecular weight transcripts complementary to the rp 51 and the rp 52 plasmids is increased. Analysis of the rp 51 plasmid transcripts reveals that the temperature-induced higher molecular weight transcripts differ from the mature rp 51 mRNA by the presence of an intron. This observation and the kinetics with which the concentration of the various rp 51 transcripts change after a temperature shift suggest that the effect of rna2 may be at the level of processing of rp mRNA.

Introduction

The biosynthesis of ribosomes is an important aspect of cellular growth and regulation. Although much is known about the regulation of ribosome biosynthesis in Escherichia coli (for example, see Yates and Nomura, 1980), relatively little is known about the details or mechanisms of such regulation in eucaryotes. Yeast is an ideal eucaryotic organism for the study of regulation because it is amenable to both physiological and genetic manipulation. Results from genetic manipulation of the conditional lethal rna mutants have proved interesting. These mutants were originally isolated and identified as being defective in RNA synthesis at the nonpermissive temperature (Hartwell et al., 1970). Recently, a more detailed picture of their effects on cellular metabolism has emerged. The mutant rna1 (ts168) is thought to prevent transport of mRNA from nucleus to cytoplasm (Hutchinson et al., 1969; Shiokawa and Pogo, 1974) and also to cause the accumulation of tRNA precursors at the nonpermissive temperature (Hopper et al., 1976; Knapp et al., 1978). The other mutants identified in the original screen, rna2 through rna7, are considerably different. At the nonpermissive temperature, ribosomal RNA synthesis occurs normally, but the processing of ribosomal RNA is defective (Warner and Udem, 1972; Ghulman and Warner, 1970). In addition, there is a marked effect of these mutations on the levels of almost all ribosomal protein mRNAs (Warner and Udem, 1972). At the nonpermissive temperature, these mRNA species decrease rapidly in concentration to approximately 10% of the wild-type levels (Warner and Gorenstein, 1977). The effect is specific in that the level of nonribosomal protein mRNAs and total messenger RNA is not appreciably changed. The decrease in ribosomal protein mRNA, as assayed by pulse-labeling of proteins in vivo or by isolation of mRNA and translation in vitro, is quite rapid (Gorenstein and Warner, 1976; Warner and Gorenstein, 1977). The data are consistent with the hypothesis that at the nonpermissive temperature ribosomal protein mRNA transcription is significantly reduced; the pre-existing ribosomal protein mRNA decays stochastically (Warner and Gorenstein, 1977).

In an effort to understand in more detail the way in which ribosome metabolism is regulated, we have cloned several genomic segments of yeast DNA, each of which contains a single ribosomal protein gene (Woolford et al., 1979). In this study we report the use of these clones to examine the mechanisms by which the rna mutations affect this coordinately controlled set of genes. The data suggest that in a mutant rna2 background, the processing of at least one ribosomal protein mRNA is defective. They also suggest regulatory mechanisms that might explain the response of ribosome metabolism to theo6 mutations.

Results

Mutant rna2 Affects the Levels of Transcripts from Ribosomal Protein Genes

We have previously identified five clones containing ribosomal protein genes. One of these contains repeated sequences and has not been analyzed further (Woolford et al., 1979). Each of the other four clones contains a single ribosomal protein structural gene and codes for a single moderately abundant mRNA as assayed by in vitro protein synthesis. Two of these clones, pY11 and pY19, which codes for rp 51, and pY18 and pY22, which codes for rp 52, are representative of most ribosomal protein genes in that the levels of their complementary mRNAs, as assayed by in vitro trans-
lation, are sensitive to the *rma2* mutation. The other two, *pY10-78* and *pY11-40*, are complementary to *rp39* mRNA (there are at least two genes for this protein), and the mRNA levels for this ribosomal protein are unaffected by the presence of the *rma2* mutation. These results suggest that each of the clones, when used as a probe on "Northern" gels, should hybridize to a single major mRNA species. Furthermore, they can be used to predict the levels of these four mRNAs in different genetic backgrounds. The data shown in Figure 1 confirm these predictions. In all cases, the four clones hybridize to a single, rather broad RNA band. Moreover, the behavior of the RNA bands in response to a temperature shift in a number of genetic backgrounds is consistent with the previous studies cited above.

After one hour at the nonpermissive temperature in an *rma2* background (ts368), the major RNAs complementary to *pY11-138* and *pY13-86* are significantly reduced in amount (Figures 1A and 1B, lanes 3 and 4). In contrast, the two other clones (pY10-78 and pY11-40) hybridize to a major mRNA, the concentration of which is not reduced by incubation of ts368 at the nonpermissive temperature (Figures 1C and 1D, lanes 3 and 4). The incubation of the strain RNA2- (a single-site revertant of ts368) for 60 min at 36°C has no major effect on the levels of any of the ribosomal protein mRNAs (lanes 1 and 2). The results of this experiment therefore corroborate and extend data obtained by in vitro translation or by in vivo labeling of ribosomal proteins (Gorenstein and Warner, 1976; Warner and Gorenstein, 1977)—that is, only the concentration of *rma2*-sensitive ribosomal protein mRNAs is affected by the presence of the *rma2* locus.

Also shown on Figure 1 is RNA isolated from ts136 (rma1) inculuated at both the permissive and nonpermissive temperature for 60 min (lanes 5 and 6). The data indicate that both *rma2*-sensitive and *rma2*-insensitive ribosomal protein mRNA sequences are significantly reduced in an *rma1* background after incubation at the nonpermissive temperature (lane 6). The data are consistent with the notion that *rma1* has a general effect on RNA metabolism, while *rma2* is specific for a subset of ribosomal protein genes. The effect of the presence of both *rma1* and *rma2* (a haploid double mutant strain containing both mutants *rma1* and *rma2*) is shown in lanes 7 and 8. The RNA levels change in a manner similar to those found in an *rma2* background but unlike those found in an *rma1* background.

When the gels shown in Figure 1 are exposed for a longer time, additional RNA bands are visible. In the case of the plasmids that code for *rma2*-sensitive ribosomal proteins (pY11-138 and pY13-86), some of the higher molecular weight RNAs increase in concentration in ts368 at the nonpermissive temperature as compared to ts368 at the permissive temperature (see below). Because this increase is much more dramatic for pY11-138 than for pY13-86 complementary RNAs, we concentrated our efforts on pY11-138 and constructed a number of subclones to map the RNA species on this plasmid. In addition, a recombinant phage containing pY11-138 sequences was purified (Figure 2).

In Figure 3, lanes 1–12, show the hybridization of four of these recombinant DNA molecules to pA+ RNA isolated from *rma2* cells after incubation for 60 min at the permissive or the restrictive temperature. As expected, the recombinant phage *hybridizes* to a large number of bands, presumably because there are on average five to seven mRNAs encoded by a 16 kb piece of yeast DNA (lanes 1 and 2) (Hereford and Rosbash, 1977; Kaback et al., 1979). As expected, the original plasmid pY11-138 hybridizes to a subset
of these bands (lanes 3 and 4). In this case the bands controlled by the *ma2* locus are easily visible and indicated by the arrow. These bands can also be visualized by hybridization to the phage DNA (lanes 1 and 2), although the effect is less marked in this case, presumably because a smaller fraction of the phage DNA, as compared with the plasmid DNA, is complementary to the mRNAs. The major (most frequent) R loop on *py11-138* was previously localized to the region indicated by the solid rectangle in Figure 2 (Woolford et al., 1979). This region should be contained within subclone 2. Consistent with this notion is the observation that subclone 2 can be used to hybrid-select the mRNA for rp 51 (data not shown).

When subclone 2 is hybridized to the same gel (lanes 5, 6, 9 and 10), only the bands controlled by the *ma2* locus are visible. There are at least two and perhaps three higher molecular weight RNAs that increase in concentration in response to the elevated temperature, and two or three lower molecular weight mRNAs that decrease. These RNAs hybridize poorly but detectably with subclone 1 (data not shown) and do not hybridize with subclone 3 (lanes 7, 8, 11 and 12).

These RNAs therefore are probably coded for largely by the information contained in subclone 2, consistent with the original localization of the gene. Plasmid *py13-86* has not been subcloned, but hybridization of this plasmid to the same RNA shows a similar phenomenon—that is, a decrease in the major mRNA and a simultaneous increase in a higher molecular weight RNA(s) upon incubation of *ma2* at the nonpermissive temperature (Figure 3, lanes 13 and 14). It is possible that all of these transcripts also originate from the same subregion of *py13-86* as in *py11-138*.

The data also suggest that all of the *py11-138* complementary RNAs that are affected by incubation at 36°C in *ts368* map to subclone 2. The use of probes that cover larger regions of the surrounding DNA (lanes 1 through 4) show no effect, either quantitative or qualitative, on other transcripts that do not hybridize to subclone 2. A direct demonstration of this phenomenon is in lanes 7 and 8 and 11 and 12, which show hybridization of subclone 3 to the same gel. The RNAs that hybridize to subclone 3 are not affected by incubation at the nonpermissive temperature, consistent with the data in other lanes of this figure. These mRNAs are presumably at too low a concentration to have been detected in our previous electron microscopic observations (Woolford et al., 1979). All these observations suggest that the transcripts affected by the *ma2* locus come from a region near (or within) the rp 51 gene; the flanking transcripts appear unaffected.

**RNAs That Increase in Concentration in the Presence of *ma2* Are Precursors**

As mentioned above, "Northern" gel analysis suggested that a small fraction of the rp 51 gene is within subclone 1. Preliminary DNA sequence data to the left and to the right of the Sal I site (which forms the border between subclone 1 and subclone 2) and S1 mapping (Berk and Sharp, 1978) suggested further that subclone 1 contained the 5' end of the rp 51 gene (data not shown). Consequently, DNA was labeled at the Bgl II site (Figure 4), was hybridized to RNA, and was analyzed by S1 mapping. The data shown in Figure 4 are similar to the "Northern" gel analysis presented above, demonstrating that the concentration of the higher molecular weight RNAs is markedly elevated and that the mature mRNA is depressed by incubation of *ma2* cells at the nonpermissive temperature. Since the end-labeled DNA that hybridized to both precursor and mature RNA species
is resistant to S1 digestion, these two sets of transcripts must overlap. The sizes of the large DNA fragments protected with 34°C RNA are indistinguishable on neutral and denaturing gels (Figure 4, lanes 2 and 6), suggesting that no introns exist in the higher molecular weight RNAs. Furthermore, these sizes (~900 bp) are similar to the sizes of the precursor RNAs as measured on CH3HgOH gels (allowing for poly(A) tails; Figure 3), placing the Bgl II site near the 5' end of the gene and therefore require that the 5' exon be rather small (~50 bp). The locations of these RNAs are presented in Figure 4A, the details of which, including the nature of the RNA heterogeneity, will be the subject of a subsequent report (J. Teem and M. Rosbash, experiments in progress). These and other S1 mapping experiments confirm that the higher molecular weight RNA(s) and the mature rp 51 mRNA(s) are both transcribed from the same region of the yeast genome and that the former are precursor(s) to the latter. Our previous electron microscopic observations did not reveal an intron (Woolford et al., 1979), presumably because the 5' exon is quite small.

Comparison of rn2 with rn1

Incubation of the temperature-sensitive strain ts136 (rn1) at the nonpermissive temperature has been shown to affect the processing of tRNA precursors (Hopper et al., 1978; Knapp et al., 1978). It was therefore of interest to compare the effect of rn2 on the accumulation of rp 51 precursor RNAs with the effect of rn1. The data in Figure 5 show the rate of decrease of rp 51 mRNA after a shift-up to the nonpermissive temperature in the three strains, rn2, rn1 and rn1/2 (the haploid double mutant). The rate of decrease in rn2 is similar to the rate of decrease of ribosomal protein mRNA as assayed by in vitro protein synthesis by Warner and Gorenstein (1977). It is considerably slower in rn1, suggesting that the mechanisms by which the mature mRNA levels decrease in the two mutant strains may be different. This is consistent with the data in Figure 1, which show that rn1 affects both rn2-sensitive and rn2-insensitive mRNAs. The rate of decrease in the double mutant (Figure 5, lanes 7, 8, and 9) resembles closely the rate in rn2 and is therefore unlike the rate in rn1; as in Figure 2, it appears that rn2 is epistatic to rn1.

Figure 6 shows the response of the precursor RNA to temperature in strains rn1 and rn1/2 (lanes 7–12). Some precursor can be seen with rn1 at 23°C (lane 7), although it is relatively minor compared to the levels induced by 36°C incubation with rn2 (lanes 4, 5, and 6). Some changes are visible after shifting ts136 to 36°C (lanes 8 and 9), but little or no accumulation is visible after 60 min at 36°C (lane 9).

Comparison of rn2 with RNA2+

Warner and Gorenstein (1977) have shown that incubation of a wild-type strain at 36°C causes a transient decrease in the level of rp mRNAs, which is similar to the initial decrease in ts368. In contrast to ts368, however, the wild-type strain recovers such that, after 60 min at 36°C, the levels of rp mRNAs are
normal. To investigate the difference between these two phenomena, we have compared the effect of temperature on rp 51 transcripts between the temperature-sensitive mutant strain rna2 and the single-site revertant, strain RNA2+ (Figure 7). Both strains initially undergo a similar decrease in the amount of rp 51 mRNA in response to elevated temperature; however, the RNA2+ strain recovers by 60 min to near control levels, while the rna2 strain continues to decrease. The response of strain RNA2+ resembles the transient effect of temperature on ribosomal protein mRNA levels in the wild-type strain A364A, previously described by Warner and his colleagues. The appearance of the precursor RNAs can be detected in lanes 7 and 8, but no comparable levels of precursor are visible in RNA from the temperature-insensitive strain (compare lanes 3 and 7). This difference can be readily seen in Figure 6, lanes 1–6, in which a long exposure of a similar experiment is shown. Even with this increased sensitivity, no detectable increase in the levels of the precursor RNA takes place upon incubation of RNA2+ at 36°C. The absence of the "precursor temperature response" is particularly notable in lane 2, which contains RNA extracted from RNA2+ after 20 min at 36°C, the time at which the mature mRNA level reaches a minimum (Figure 7; and Warner and Gorenstein, 1977). The dramatic difference between rna2 and RNA2+ in the levels of the precursor RNA after a shift-up to the nonpermissive temperature suggests that the biochemical mechanism(s) responsible for the long-term (>1 hr) decrease in rp mRNA levels in rna2 cells is related to the increase in precursor RNA concentration and therefore different from the mechanism(s) responsible for the transient decrease in RNA2+ or wild-type cells. This notion is consistent with the fact that rp 39 mRNA and other rna2-insensitive ribosomal protein mRNAs undergo a transient decrease in wild-type cells (Warner and Gorenstein, 1977).

Discussion

The control of ribosome synthesis in eukaryotes has received little attention by comparison to E. coli. The advent of recombinant DNA technology makes possible detailed studies on the mechanisms by which the regulation of ribosome synthesis takes place. Using the ribosomal protein genes we have cloned, we have undertaken a preliminary study of the effect of temperature on the levels of ribosomal protein mRNAs in the temperature-sensitive rna mutants (Hartwell et al., 1970). The experiments verify results previously ob-
transcription of total mRNA (Gorenstein and Warner, 1977). After 60 min at the nonpermissive temperature in the temperature-sensitive mutant ts368 (ma2), the concentrations of rp 51 and rp 52 mRNAs, as assayed on "Northern" gels, are greatly decreased. In contrast, when the two clones complementary to rp 39 mRNA (rp 39 is one of the few ribosomal proteins that are insensitive to the RNA2 locus) are used as probes, no substantial effect of ma2 is observed (Figures 1C and 1D). Six other nonribosomal protein gene clones have also been used on these gels, with results identical to those obtained with the clones complementary to rp 39 mRNA (data not shown). All of these data suggest that the effect of the ma2 locus is relatively specific for ribosomal protein mRNAs.

In the presence of the mutant ma2 gene product, transcription or some posttranscriptional step is sensitive to ma2 (Figures 2 and 3). These data are consistent with recent unpublished data which suggest that no additional ribosomal proteins reside in the immediate vicinity of the other three plasmids used in this study, pY19-86, pY10-79, pY11-40 (J. L. Woolford, Jr., and M. Rosbash, manuscript in preparation). They are also consistent with recent studies which show that all four of these plasmids map to unlinked regions of the yeast genome (L. Hyman, unpublished observations).

The data described above suggest that ma2 does not operate by inhibiting transcription from a region of chromatin, thereby affecting a group of linked genes. The effect must be on individual rp gene sequences, either at the transcriptional or the posttranscriptional level. The appearance of an intron-containing precursor RNA shortly after shifting to the nonpermissive temperature is consistent with this interpretation since the precursor RNA (by definition) maps to the same region of DNA as the mature rp 51 mRNA. The dramatic increase in precursor RNA concentration, paralleled by a decrease in the concentration of the mature mRNA, points toward a posttranscriptional block as being responsible for the decrease in rp 51 mRNA. Consistent with this view is the observation that the precursor RNA is transcribed at 23°C in ts368 and can be "chased" into mature rp 51 mRNA when cells are shifted back to 23°C (S. Bromley, L. M. Hereford, M. Rosbash, manuscript in preparation).

The findings presented in this communication are reminiscent of the effect of the rna1 mutation on intron-containing tRNAs (Hopper et al., 1978; Knapp et al., 1978). It was therefore of interest to compare ts136 with ts368. The data indicate that, in contrast to ts368, the rp 51 precursor does not accumulate upon incubation of ts136 at the nonpermissive temperature (Figure 6). Moreover, they demonstrate that the levels of both rna2-sensitive and rna2-insensitive rp mRNAs decrease in ts136 (Figure 1) as do the levels of all (six) other nonribosomal mRNAs examined to date (data not shown). In the case of rp 51 mRNA, we have also compared the rate of decrease in ts136 and ts368 and found the rate to be considerably faster in ts368 (Figure 5). While one or more of these observations may be due to allele-specific effects, the rp 51 mRNA decrease and rp 51 precursor RNA accumulation probably are not, since they occur in a similar manner in mutants ma6 and ma8 (data not shown).

Taken together, these facts suggest that rna1 and ma2 are phenotypically distinct mutations.

Involvement of the RNA1 gene product in transport of RNA from nucleus to cytoplasm has been proposed (Hutchison et al., 1969; Shiokawa and Pogo, 1974). The decrease in mRNA levels in ts136 is therefore somewhat unexpected and suggests that the effects of this mutation are more complex than previously indicated. Even more surprising is the phenotype of...
the ma1/2 double mutant. The rna2 mutation is epistatic to ma1 (Figures 1, 5 and 6), even for rna2-insensitive RNAs (Figure 1 and data not shown)—that is, the levels of rna2-insensitive RNAs do not decrease upon incubation of the double mutant strain at the nonpermissive temperature. These data suggest that these two rna mutations, although phenotypically distinct, interact in some way.

It has been proposed that the RNA2 locus is specifically involved in the control of ribosomal protein synthesis (Warner and Gorenstein, 1977). The data presented in this report suggest that this control might take place at the posttranscriptional level, although confirmation of this control mechanism awaits the examination of many more ribosomal protein genes and additional experimental support. The observations that the rp 51 gene has an intron and that the processing of the rp 51 intron-containing precursor transcript is defective in ts368 raise an intriguing possible explanation for the specificity of this mutation for ribosomal protein genes. Perhaps in the absence of the RNA2 gene product the processing of all intron-containing transcripts is defective? If this were the case, the mutant phenotype would appear specific for ribosomal proteins because these genes constitute a substantial fraction of all intron-containing yeast genes. Experiments to distinguish between these two hypotheses are currently in progress.

**Experimental Procedures**

**Yeast Strains and Cell Growth**

The mutant ma2 (ts368) was obtained from John Warner; ma1 (ts136) was obtained from Jim Haber (see Harshwell et al., 1970). RNA2 was selected as a spontaneous (temperature-insensitive) revertant of ts368 by C. Biffo in this laboratory several years ago. The reversion site is at or very near the ma2 locus since, upon mating the RNA2 - strain with a wild-type strain, there was no segregation of temperature sensitivity for 30 four-spored ascii. This strain provides us with the croser control for comparison with ma2. A haploid strain containing both ma1 and ma2 mutant loci, ma1/2, was constructed for us by Nancy Pearson. Cells were grown at 23°C as in Woolford et al., 1979. For shift-up experiments, cells growing at 23°C were rapidly mixed with an equal volume of medium prewarmed to 42°C and then incubated at 36°C.

**RNA Extraction**

Cells were rapidly chilled in an ice-water bath. RNA was extracted as in Herford and Rosbash, 1977 or in Herford et al., 1981. The RNA was washed with 3 M NaAc, resuspended in SDS buffer (0.5% SDS, 0.1 M NaCl, 0.01 M Tris, 0.001 M EDTA [pH 7.4]) and the optical density was determined. Poly(A) RNA was prepared from "matched sets" of cells (for example, as for the experiment in Figure 5) by taking the same amount of RNA from each preparation and passing it over oligo(dT)-cellulose (T-2, Collaborative Research) once. Total RNA or poly(A) RNA was precipitated with 2.5 vol ethanol, washed twice with 70% ethanol and resuspended in water at an estimated concentration of 1 mg/ml. The actual RNA concentration of these RNA solutions was determined by subtraction of an aliquot and measurement of the optical density.

**Bacterial Plasmids and Phages**

The plasmids pY11-138, pY13-86, pY10-78 and pY11-40 have been previously described (Woolford et al., 1979). We detected 11-135s by plaque hybridization (Benton and Davis, 1977) with nick-translation pY11-138 to a partial E. coli library in Charon 4A (Blattner et al., 1978), constructed for us by U. Schafer. Recombinant phages (12,000) were screened, from which ten identical 11-138 phages were identified; Subclones of pY11-138 were generated into pbR322 as in Barnett et al. (1980). Plasmid DNA (Maniatis et al., 1978) and plasmid DNA (Maniatis et al., 1978) were prepared by standard procedures.

"Northern" Gels

The protocol for the electrophoresis and treatment of RNA gels is as described in Golden et al., 1980. When the data were quantified, the gels were exposed to Kodak XRS film preexposed to an optical density of 0.1-0.2. Exposures were made at -80°C with Dupont Cronex Lightning Plus intensifying screens. The autoradiographs were quantitated optically with a Joyce-Loeical recording densitometer. To correct for differences in RNA concentration (which were rarely >1%) we determined the amounts of 18S and 26S ribosomal RNA by scanning a negative of the stained gel. Recombinant DNAs were labeled by nick translation with either 32P-dATP or 32P-dATP (New England Nuclear) and prepared as in Golden et al., 1980.

**S1 Mapping**

Five micrograms of pY11-138, digested with both Bgl II and Kpn I, were labeled at the two ends of the single Bgl II sites with reverse transcriptase, 32P-dATP and cold dCTP (which does not label Kpn I sites). Digested DNA (5 μg) was incubated for 60 min at 42°C in a final volume of 10 μl. The reaction mix was as in Wahl et al., 1978, without actinomycin, oligo(dT), RNA, dCTP and TTP. Nonradioactive dCTP was at 100 μM, and radioactive 32P-dATP was at 50 μCi/10 μl (approximately 10 μM). Approximately 20 units (or a saturating amount) of reverse transcriptase were added. DNA was ethanol-precipitated twice and loaded on a 1% agarose gel. The appropriate radioactive Bgl II-Kpn I fragment was isolated by electroelution, purified by DEAE-cellulose chromatography, ethanol-precipitated and stored in TE (0.01 M Tris [pH 8.3], 0.001 M EDTA). RNA-DNA hybridization reactions were performed in a volume of 30 μl containing 5% (w/v) denatured tRNA, 0.1 M NaAc, 0.1 M NaCl, 0.01 M Tris [pH 8.3], 0.01 M L-lleA and 0.4 M NaCl and incubated for 3 h at 52°C. Approximately 10,000 cpm of DNA fragment were hybridized with 5 μg poly(A) RNA from rna2 cells grown at 23°C or shifted to 34°C for 1 hr (or no RNA). Hybridization reactions were diluted 10 fold into S1 buffer (Berk and Sheru, 1970) and digested 30 min at 37°C with 30 units S1 (Bethesda Research Laboratories). The nucleic acid was ethanol-precipitated with 10 μg carrier rRNA and resuspended in TE buffer. Equal aliquots from each hybridization were analyzed on nondenaturing 4.5% acrylamide-urea gels (Maniatis et al., 1975). Equal aliquots were also digested for 30 min at 37°C with approximately 200 μg/ml RNAase in TE buffer, ethanol-precipitated and analyzed on denaturing 7 M urea 4.5% acrylamide TBE gels (Maniatis et al., 1975).

**Restriction Enzyme Digestion and Agarose Gels**

Restriction enzymes were purchased from either Bethesda Research Laboratories or New England BioLabs and used as recommended by the supplier. Neutral agarose gels were as in Woolford et al., 1979.

**Biobehazard Considerations**

All recombinant DNA-containing strains and phages were propagated under NIH P2-EXK1 containment conditions.

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