Polyadenylic Acid Sequences in Yeast Messenger Ribonucleic Acid*

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SUMMARY

Polyadenylate sequences are found covalently linked to yeast mRNA molecules. The poly(A) sequences are relatively homogeneous in size and have an average chain length of about 50 residues. They are located at the 3' end of the mRNA molecule. Our data suggest that some mRNA molecules may not contain poly(A) sequences and that no poly(A) sequences are present in rRNA. The occurrence of poly(A) sequences in the mRNA molecules of mammals and yeast suggests that this may be a general feature of eucaryotic organisms.

The demonstration that relatively homogenous polyriboadenyl acid (poly(A)) sequences between 150 and 200 nucleotides in length are found covalently linked to RNA in the rapidly labeled, heterogeneous nuclear RNA and the messenger RNA (mRNA) fractions of mammalian cells (1-4), stimulated us to examine the situation in a lower eucaryotic organism, Saccharomyces cerevisiae. Yeast was selected as the organism for this study because it is one of the simplest eucaryotic organisms, with only a few times more DNA than Escherichia coli (5), yet it has the cellular complexity associated with eucaryotic organisms and has been shown (6) to contain poly(A) sequences. The basic kinetic parameters of RNA synthesis in yeast have been described (7-12) and resemble in outline the kinetic parameters of RNA synthesis in mammalian cells (13). Further, a number of temperature-sensitive, conditional-lethal mutants defective in RNA metabolism have been isolated and characterized in yeast (7, 9, 14, 15). These mutants can be used to examine the pathways of RNA metabolism, including control mechanisms that operate in the specifically altered patterns of RNA metabolism in the mutants at the nonpermissive temperature.

The poly(A) content of the yeast RNA fractions has been measured by the hybridization of poly(A) sequences to polydeoxythymidylylate oligomers covalently bound to cellulose (1, 16). This sensitive method quantitatively binds poly(A) sequences of chain length longer than about 20 nucleotides. Our results indicate that yeast polysome RNA contains poly(A) sequences attached to a rapidly labeled, polydisperse RNA fraction that previous studies have identified as mRNA (7-9). Yeast rRNA appears to contain less than 0.04% of poly(A) sequences long enough to bind to polydeoxythymidylylate cellulose (poly(dT)-cellulose).

MATERIALS AND METHODS

Strains—The parent yeast strain S. cerevisiae A804A and the origin of the mutants selected from it and composition of the media (YM-1, YM-5, and YEFP-D-TAU plates) were described previously (14, 15).

Chemicals—[8-14C]Adenine (specific activity 41.3 mCi per mmole), and [5-3H]uridine (specific activity 20.7 Ci per mmole), were purchased from Schwarz BioResearch, Inc. [G-3H]Adenine (specific activity 4 Ci per mmole), was purchased from the New England Nuclear Corp.

Buffers—Buffer P contains 0.03 M MgCl₂, 0.1 M NaCl, 0.01 M Tris, pH 7.4. Buffer S contains 5 × 10⁻⁴ M MgCl₂, 0.1 M NaCl, 0.01 M Tris, pH 7.4. Buffer A contains 0.2% sodium dodecyl sulfate, 0.1 M NaCl, 0.01 M EDTA, 0.01 M Tris, pH 7.4.

Cell Labeling and Fractionation—Cells were grown in YM-1 medium to a concentration of 1 × 10⁶ cells per ml. Spheroplasts were prepared from these cells as described (7). The spheroplasts were grown 2 hours in YM-5 containing only 2 mg per ml. E. Edmonds, unpublished data.

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litter of adenine and uracil at 23°, with 0.5 m MgSO₄ added for osmotic stabilization. The spheroplasts were labeled by adding 0.125 μCi per ml of the appropriate ¹⁴C-nucleoside or base or 1.25 μCi per ml of the appropriate ³²P-nucleoside or base to the media. The incorporation was stopped by chilling. The spheroplasts were collected by centrifugation, lysed, and layered on a 10 to 50% sucrose gradient in Buffer P as described (9, 15) for the preparation of polysomes.

RNA Analysis—The RNA samples were prepared by extraction with phenol as previously described (17), with the modification that occasionally a 1:1 (v/v) mixture of chloroform and phenol was substituted for phenol. This substitution does not affect the poly(A) content of the isolated RNA. Polyacrylamide gel electrophoresis of RNA has been described (18), as has the sedimentation analysis of the RNA samples (1). The ribosomal subunits were prepared from the polysome regions by centrifuging the pooled polysome fractions for 3 hours at 25,000 x g. The pellet was resuspended in Buffer S and layered on a 10 to 30% sucrose gradient containing Buffer S. The gradients were centrifuged at 160,000 x g for 11 hours (8).

Polyadenylate Acid Analysis—Poly(A) was isolated, detected, and measured as described previously (1, 2), using digestion with pancreatic and T₁ ribonucleases to release poly(A) from poly(A) RNA prior to assay by binding to polydeoxythymidylate cellulose (poly(T)-cellulose). RNA binding to poly(T)-cellulose was determined by a modification of the method of Nakazato and Edmonds (19). The RNA sample dissolved in 2 ml of Buffer A was added to polydeoxythymidylate cellulose (250 mg) and the mixture was shaken at room temperature for 1 hour. The cellulose was collected by filtration and washed 10 times with 3-ml portions of Buffer A. The pooled washes contain the unbound RNA fraction. The poly(T)-cellulose was then eluted at 60° with Buffer A minus NaCl, once with 2 ml and six times with 1 ml. The pooled eluates contain the bound RNA fraction. As a final step the pooled fractions were filtered through Millipore filters to remove any poly(T)-cellulose present before the RNA was analyzed further. At the low ionic strength of the eluate, and in the presence of sodium dodecyl sulfate, poly(A) is not bound to the Millipore filter (4).

Determination of Chain Length and Location of Poly(A) Sequences—Poly(A) was isolated from the polysomal RNA of yeast labeled 10 min with [³²P]adenine as described (1). One-half of the poly(A), approximately 40 μg, was dissolved in 0.07 ml of 0.01 m Tris, 0.3 m MgCl₂, pH 7.5, containing 5 units of electrophoretically purified alkaline phosphatase (Worthington Biochemical Co.) and was incubated 40 min at 37°. The incubation was terminated by the addition of 0.01 ml of 0.16 m EDTA, 0.8% sodium dodecyl sulfate, pH 7, plus 0.02 ml of ³²P-labeled HeLa cell transfer RNA (approximately 0.05 mg). The remainder of the poly(A) was dissolved in the same manner but without the addition of alkaline phosphatase and incubation. Both poly(A) samples were then subjected to electrophoresis on 10% acrylamide gels as described (1, 18) and shown in Fig. 5. After elution of the gel fractions with 0.5% sodium dodecyl sulfate, the fractions containing the peak of ³²P-labeled poly(A) were pooled, carrier polynucleoside acid was added (100 μg), and the RNA was precipitated with ethanol prior to hydrolysis with KOH (2). The hydrolysates, adjusted to pH 9, were chromatographed with nonradioactive adenosine and 2',3'-adenylic acid on a column, 30 x 0.5 cm, of Dowex 1-formate resin (20). The ³²P eluting with each of the three marker peaks was then determined by scillation counting.

RESULTS

Poly(A) Sequences in Yeast RNA—Poly(A) sequences are found in the total RNA extracted from yeast spheroplasts (Table I). The data in Table I indicate that the proportion of poly(A) sequences in the total yeast RNA varies with the metabolic state of the cell. Spheroplasts grown at either 36° or 23° and labeled 10 min have the same proportion of poly(A) to total RNA synthesis, while spheroplasts grown at 33° and shifted to 36° undergo a transient increase in the proportion of poly(A) to total RNA synthesis. This latter effect may result from a transient decrease in rRNA synthesis previously observed in such a temperature shift (12).

To identify the RNA species that contain poly(A) sequences, yeast spheroplasts were labeled for 15 min at 23° with [¹⁴C]adenine and [³²P]uridine prior to cell lysis and analysis for polysomes by sucrose gradient centrifugation. Fig. 1A shows the RNA-labeling pattern of the polysomes under these conditions. Little difference is observed between the labeling pattern with adenine and uridine, so no sedimenting component appears to be unusually rich in RNA labeled with adenine. Each gradient was divided into three regions: the top of the gradient, which contains RNA species sedimenting at less than 10 S, predominately 4 S RNA; a second region which includes the free ribosomes; and the polysome region which usually contains over 80% of the total cellular RNA. Previous studies have shown (7) that virtually all of the cytoplasmic RNA is displayed in a gradient analysis such as this. Fig. 1B shows the labeling pattern obtained with the same precursors and conditions when the labeling time is extended to 1 hour. At 1 hour a much smaller proportion of the total newly synthesized RNA is present in the components found in the free ribosome region. The RNA species sedimenting in this region of the gradient are known to include newly synthesized rRNA (21) and, as will be discussed in a future communication, also newly synthesized mRNA. The percentage of the RNA labeled with adenine present as poly(A) sequences in each of the three gradient regions is also indicated in Fig. 1. While the over-all radioactivity in the polysome region increased over 10-fold between 15 and 60 min of labeling, the percentage of poly(A) in this region decreased from 3.4 to 0.8%. Yeast polysomal RNA has been shown (7) to include unstable message RNA, which becomes labeled immediately after the addition of radioactive nucleotides and turns over with a half-life of about 20 min. Ribosomal RNA in the polysomes becomes labeled at a constant rate after a lag of approximately 5 min (11). The

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total cpm in poly(A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A364A spheroplasts grown at 23° and labeled 10 min at 23°</td>
<td>2.4</td>
</tr>
<tr>
<td>2. A364A spheroplasts grown at 36° and labeled 10 min at 36°</td>
<td>2.7</td>
</tr>
<tr>
<td>3. A364A spheroplasts grown at 23°, shifted to 36° for 10 min, and then labeled during the second 10-min interval at 36°</td>
<td>4.0</td>
</tr>
</tbody>
</table>
FIG. 1. Sedimentation analysis and poly(A) content of yeast RNA in polysomes and more slowly sedimenting cytoplasmic components. Spheroplasts were labeled with [14C]adenine and [3H]uridine at 23°, as described under "Materials and Methods," for a period of (A) 15 min and (B) 60 min. The cells were used to prepare cytoplasmic extract which was then analyzed for polysomes by sucrose gradient sedimentation as described. Aliquots of each gradient fraction were analyzed for radioactivity in total RNA, and the remainder of the fractions was pooled into three portions for poly(A) analysis as indicated by arrows in the figure. The percentage of the total [14C]adenine-labeled RNA as poly(A) is indicated in each of the three gradient regions. Sedimentation is to the right, and from left to right the three designated regions of the gradients contain: the top of the gradient, ribosomal subunits and free ribosomes, and polysomes. The absorbance at 260 nm is indicated in addition to the radioactivity in RNA labeled with W (••••) and 3H (---).

Labeling kinetics of the poly(A) in polysomal RNA suggests that it is associated with unstable RNA, rather than with rRNA, and is consistent with an association of the poly(A) with message RNA.

Polysome Association of Poly(A)-containing RNA—To exclude the possibility that the poly(A)-containing species in the polysome region might be fortuitously associated with the polysomes, we examined RNA metabolism in the temperature-sensitive mutant prt-1, which has a defect in an initiation step in protein synthesis (22). At the nonpermissive temperature, 36°, almost all the RNA labeled with [14C]adenine in 15 min at 23° sediments with the free ribosomes, including that associated with polysomes before the temperature shift. Table II indicates that most of the labeled poly(A) sequences in mutant prt-1 are found in the polysomes at the permissive temperature and in the region with free ribosomes at the nonpermissive temperature, paralleling the behavior of the rest of the labeled RNA. Thus, the poly(A)-containing RNA sequences are not present in the polysome region if the polysomes are not intact.

In a related experiment we mixed unlabeled spheroplast extracts, from mutant prt-1 at 23°, with [14C]adenine-labeled spheroplast extracts made from this mutant at 36° to determine whether unlabeled intact polysomes could carry [14C]adenine-labeled RNA into the polysome region. No transfer of labeled RNA to the polysome region was observed. We conclude from these experiments that the poly(A)-containing RNA molecules found in the polysome region are an integral part of the polysome structure. In yeast there does not appear to be a substantial amount of RNA co-sedimenting with polysomes which is not intrinsically associated with their structure (7-9).

Poly(A) Sequences Are Found in mRNA Fraction—To determine which polysomal RNA species contain poly(A) sequences, the polysomes from cells labeled for 15 min with [14C]adenine and [3H]uridine were collected by sedimentation and were dissociated into 40 and 60 S ribosomal subunits and a polydisperse mRNA-protein complex (8) by solution in Buffer S. The mixture was then analyzed by sedimentation on a sucrose gradient, Fig. 3. The slower sedimenting, polydisperse mRNA fraction (8) contains almost all the poly(A) sequences present in the

![Fig. 2. Sedimentation analysis of cytoplasmic extracts from mutant prt-1 at the permissive (23°) and nonpermissive (36°) temperatures. Spheroplasts of mutant prt-1 were prepared and labeled with [14C]adenine and [3H]uridine at 23° for 15 min. A sample (A) was removed and cytoplasmic extract was prepared for polysome analysis by sucrose gradient centrifugation. At this time, the rest of the sample (B) was shifted to 36° for 15 min before preparation of cytoplasmic extract for sucrose gradient centrifugation. Sedimentation is toward the right, and the peak due to free ribosomes is at Fraction 10. The absorbance at 260 nm and the radioactivity in RNA are indicated by the solid and dashed lines, respectively.](http://www.jbc.org/)

![Fig. 3.](http://www.jbc.org/)

**TABLE II**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total [14C]adenine-labeled RNA in polysome region</th>
<th>Total [3H]adenine-labeled poly(A) in polysome region</th>
<th>Total [14C]adenine-labeled RNA as poly(A) in region of Free ribosomes</th>
<th>Polysomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>prt-1 at 23°</td>
<td>55 %</td>
<td>66 %</td>
<td>2.3 %</td>
<td>3.6 %</td>
</tr>
<tr>
<td>prt-1 at 36°</td>
<td>26 %</td>
<td>25 %</td>
<td>3.1 %</td>
<td>3.0 %</td>
</tr>
</tbody>
</table>

*Details of the experiment are described in Fig. 2.

Fractions 6 to 15 of gradients shown in Fig. 2.

Fractions 16 to 40 of gradients shown in Fig. 2.
Spheroplasts labeled for 15 min with [14C]adenine and [3H]uridine as described under "Materials and Methods" were used to prepare polysomes, which were dissociated into ribosomal subunits and messenger RNA-ribonucleoprotein complexes by suspension in Buffer S, as described under "Materials and Methods." The mixture of ribosomal subunits and ribonucleoprotein complexes was then analyzed by sedimentation sucrose gradient as shown. Aliquots of gradient fractions were taken for determination of radioactivity of 14C (A- ---A) and 3H (O---O) in RNA as well as the content of 14C-labeled poly(A), indicated in Table III. Sedimentation is toward the right and the positions of the 60 and 40 S ribosomal subunits are indicated.

**Table III**

**Poly(A) content of yeast polysomal RNA species**

Details of this experiment are described in Fig. 3. The fractions were pooled, and the RNA was isolated by phenol extraction and analyzed for poly(A) content.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Adenine cpm as poly(A) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (Fractions 8 to 13)</td>
<td>6.2</td>
</tr>
<tr>
<td>2. (Fractions 24 to 30)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

polysomes (Table III), and it is again clear from comparison of the labeling patterns in Fig. 3 that no sedimenting component is particularly rich in sequences labeled with adenine. The poly(A) sequences cosedimenting with the 40 and 60 S subunits probably represent large mRNA-protein complexes. When polysomal RNA was isolated from cells labeled for several generations with [14C]adenine and given a chase of nonradioactive adenine for 2 hours, we found the RNA contained only 0.04% of its sequences as poly(A), indicating that yeast rRNA, like human rRNA (1), does not contain a significant amount of poly(A). Thus, we conclude that the poly(A) sequences in yeast polysomes are found in the polydisperse mRNA fraction.

**All Yeast mRNA Molecules May Not Contain Poly(A) Sequences** To determine whether all mRNA molecules contain poly(A) sequences, we took advantage of the technique for reversibly binding poly(A)-containing RNA sequences to poly(T)-cellulose developed by Nakazato and Edmonds (19). Strain A364A spheroplasts were labeled for 10 min with [14C]adenine and the polysomal RNA was prepared by phenol extraction and applied to a filter of poly(T)-cellulose. Fig. 4 shows the sedimentation properties respectively of the original polysomal RNA, the RNA fraction that did not bind to poly(T)-cellulose, and the RNA fraction that bound to the poly(T)-cellulose. The original polysomal RNA contains a mixture of the easily discernible 17 and 25 S rRNA species and polydisperse, rapidly labeled mRNA molecules, as expected from the data presented in Fig. 3. The RNA species bound to poly(T)-cellulose do not include the rRNA present in the original sample. The average molecular weight of the bound RNA is estimated to be about 250,000 from its sedimentation properties, in agreement with the minimum average molecular weight of unfractionated yeast mRNA determined by a different method.2 From the analyses of the bound and unbound fractions for poly(A), Table IV, the average poly(A) content of the bound mRNA is found to be 13.4%, while the poly(A) content of the unbound RNA is negligible.

The unbound RNA contains the RNA species and a substantial amount of labeled polydisperse RNA. Some or all of these polydisperse molecules, which we assume to be mRNA, may have originally contained poly(A) sequences which have been removed by the action of ribonucleases, either in vivo or...

**Table IV**

**Poly(A) content of polysomal RNA species bound and unbound to poly(T)-cellulose**

Details of the experiment are presented in Fig. 4.

<table>
<thead>
<tr>
<th>RNA Species</th>
<th>RNA Bound to Poly(T)-cellulose</th>
<th>% of Total</th>
<th>RNA Not Bound to Poly(T)-cellulose</th>
<th>% of RNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpm</td>
<td>cpm</td>
<td>% of total cpm</td>
<td>cpm</td>
<td>% of RNA cpm</td>
<td></td>
</tr>
<tr>
<td>RNA bound to poly(T)-cellulose</td>
<td>96,700</td>
<td>25</td>
<td>13,000</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>RNA not bound to poly(T)-cellulose</td>
<td>283,000</td>
<td>75</td>
<td>1,390</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>379,700</td>
<td>100</td>
<td>14,390</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

N. S. Peterson and C. S. McLaughlin, unpublished work.

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**Fig. 3.** Sedimentation analysis of the ribonucleoprotein components from yeast polysomes. Spheroplasts labeled for 15 min with [14C]adenine and [3H]uridine as described under "Materials and Methods" were used to prepare polysomes, which were dissociated into ribosomal subunits and messenger RNA-ribonucleoprotein complexes by suspension in Buffer S, as described under "Materials and Methods." The mixture of ribosomal subunits and ribonucleoprotein complexes was then analyzed by sedimentation sucrose gradient as shown. Aliquots of gradient fractions were taken for determination of radioactivity of 14C (A- ---A) and 3H (O---O) in RNA as well as the content of 14C-labeled poly(A), indicated in Table III. Sedimentation is toward the right and the positions of the 60 and 40 S ribosomal subunits are indicated.

**Fig. 4.** Sedimentation analysis of the RNA components from yeast polysomes. A364A spheroplasts were labeled with [3H]adenine for 10 min at 20°, lysed, and the polysomes were isolated by centrifugation. RNA from the polysome region was prepared by phenol extraction and tested for its ability to bind to poly(T)-cellulose as described under "Materials and Methods." Sucrose gradient sedimentation analyses are shown of (A) the original polysomal RNA sample, (B) the polysomal RNA fraction that does not bind to poly(T)-cellulose, (C) the polysomal RNA fraction that binds to poly(T)-cellulose. Sedimentation is toward the right and arrows indicate the positions of the 17 and 25 S yeast ribosomal RNA species. In Part C the ultraviolet absorption pattern is due to added HeLa cell ribosomal RNA.
during preparation of the RNA. However, significant digestion by ribonucleases during RNA preparation is unlikely. The internal ultraviolet absorption markers from the accompanying rRNA showed the expected 2:1 absorbance ratio between 25 and 17 S rRNA, as indicated in Fig. 4, while the low ratio of single ribosomes to polysomes shown in Fig. 1 and Fig. 2A also suggests that nucleases were not active in these preparations. Furthermore, the bound RNA has the average molecular weight expected of the total mRNA, whereas appreciable nuclease cleavage would presumably reduce the average molecular weight of the bound RNA. A significant contamination of the polyribosomes with labeled nuclear RNA is also unlikely; the data shown in Fig. 2 indicate that under the conditions of these experiments the bulk of the labeled RNA which sediments in the polysome region is physically associated with the polysomes per se. We conclude that the poly(A) is in mRNA molecules, but that some of the yeast mRNA may lack poly(A) sequences.

Size and Location of Poly(A) Sequences in mRNA—We determined the size and location of the poly(A) in mRNA by analyzing poly(A) labeled with [3H]adenine for the presence of adenosine at its 3' terminus. The poly(A) was isolated by the poly(T)-cellulose-binding method after its release from [3H]adenine-labeled polysomal RNA by the combined action of pancreatic and T1 ribonucleases (1). The poly(A) was further purified by electrophoresis on a 10% acrylamide gel. The electrophoresis pattern, Fig. 5, shows that the poly(A) sequences in yeast mRNA are reasonably homogeneous in chain length and considerably smaller than the poly(A) sequences found either in HeLa cell nuclear heterogeneous RNA (note arrow in Fig. 5) or HeLa mRNA (1). Purified poly(A) was obtained from elution of gel fractions containing the [3H]-labeled peak and was hydrolyzed with KOH. The alkaline hydrolysate was chromatographed on Dowex 1 resin and 29,000 cpm were recovered in 2'- and 3'-AMP versus 620 cpm in adenosine. Another sample of poly(A) treated with alkaline phosphatase prior to electrophoresis and subsequent alkaline hydrolysis gave similar results in the chromatographic analysis: 17,200 cpm in 2'- and 3'-AMP versus 335 cpm in adenosine. From these results we conclude that the poly(A) released from mRNA has no phosphate on its 3' end and must thus constitute the 3' terminus of the mRNA molecules. We conclude from the ratio of counts in AMP and adenosine that these poly(A) sequences are about 50 residues long.

The ratio of [3H] radioactivity in AMP to GMP in the bound RNA of the experiment in Fig. 4 was found to be 1.89, which is nearly the ratio of these nucleotides in yeast DNA (31 versus 17.6%, Reference 23). Thus, if the average [3H]-labeled poly(A) content of mRNA is 13.4% under the conditions of the latter experiment, Table IV, then an average poly(A) length of 50 AMP residues indicates an average mRNA length of approximately 750 nucleotides, in reasonable agreement with the estimate of 250,000 for the molecular weight, as deduced from its sedimentation properties.

DISCUSSION

These results establish that yeast mRNA molecules contain at their 3' termini sequences of poly(A) approximately 50 residues long. In addition, our data suggest that some of the rapidly labeled, polydisperse RNA molecules in yeast polysomes may not contain poly(A) sequences. Previous work (7-9) indicates that yeast polysomes contain only mRNA, rRNA, and tRNA, so the simplest interpretation of our results is that yeast mRNA may include some molecules without a poly(A) sequence. Yeast rRNA contains less than 0.05% of its sequences as poly(A) (23). Thus, if the average aH-labeled poly(A) content of yeast mRNA is 13.4% versus 335 cpm in adenosine. From these results we conclude that poly(A) and may contain none, since it does not bind to poly(T)-cellulose.

The indications that some yeast mRNA molecules contain poly(A) while some may not is interesting in view of recent findings that, although most of the mRNA molecules of animal cells contain poly(A) (19), the mRNA for histone in human HeLa cells (24) and mouse L cells does not. Thus, although the role of the poly(A) sequences is not known, it is apparently not required in the basic process of translation. It now seems more likely that the poly(A) may serve to distinguish different classes of mRNA molecules. For example, poly(A) might be involved in translational control or in regulating the half-life of mRNA molecules or in specifying the intracellular transport and localization of particular types of mRNA. We are currently examining mRNA synthesis and function under various metabolic conditions in the parent strain and conditional lethal mutants to further define the role of poly(A) in mRNA metabolism.

The occurrence of poly(A) sequences in the mRNA molecules of widely divergent organisms as mice, men (1, 3, 4), and yeast suggests that the presence of poly(A) in mRNA may be a general characteristic of eucaryotes. Furthermore, the fact that the poly(A) sequences have strikingly different chain lengths

Fig. 5. Acrylamide gel electrophoresis of poly(A) from yeast polysomes. Poly(A) was prepared as described under "Materials and Methods" and Reference 1 from the polysomal RNA of yeast labeled 10 min with [3H]adenine. The poly(A) was mixed with [3H]-labeled HeLa cell 4 S transfer RNA and 5 S rRNA and subjected to electrophoresis on a 10% acrylamide gel as described (1). A sample of [3H]-labeled poly(A) prepared from HeLa cell nuclear heterogeneous RNA (1) was run on a separate gel, under identical conditions, for comparison of electrophoretic mobilities. The electrophoretic pattern of a sample of the same yeast poly(A), pretreated with alkaline phosphatase as described under "Materials and Methods," was similar to that shown in this figure. [3H]-Labeled yeast poly(A) eluted from the appropriate fractions of these gels was used to determine the size and location of the poly(A) as described under "Materials and Methods." 33P radioactivity (---A); [3H] radioactivity (O-O).
in animal and yeast mRNA indicates that this length may be an important evolutionary characteristic of eucaryotic organisms.

Acknowledgments—We wish to thank our technicians for their aid.

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