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Length Heterogeneity in the Poly(adenylic acid) Region of Yeast Messenger Ribonucleic Acid†

Bernd Groner, Nancy Hynes, and Stephen Phillips*

ABSTRACT: The population of poly(A) chains obtained from yeast spheroplast RNA contains molecules ranging from about 20 to 60 nucleotides in length. Poly(A) containing RNA obtained from spheroplasts which have been exposed to a short pulse of [^{3}H]adenine contain label predominantly in the longest poly(A) sequences (~60 nucleotides in length). Shorter poly(A) molecules are found in cytoplasmic polyribosomes and are labeled when spheroplasts are exposed to [^{3}H]adenine for a considerably longer time. This strongly suggests that, like in human cells (Sheiness, D., and Darnell, J. E., (1973), *Nature (London), New Biol. 241,* 265) the poly(A) sequence in yeast messenger RNA becomes shorter with time. We also find that there are rapidly labeled very large poly(A) containing RNA species which are obtained from total cell extracts. The size distribution of these molecules is larger than messenger RNA obtained from polyribosomes.

Although poly(A) has been found in RNA species in many eucaryotic cells (Edmonds *et al.,* 1971; Darnell *et al.,* 1971a,b; Lee *et al.,* 1971; McLaughlin *et al.,* 1973) its metabolic role still remains to be elucidated. A function related to the production or utilization of genetic message is suggested by the presence of poly(A) in messenger RNA. Experimental evidence is available from cultured mammalian cells which indicates a nuclear function for poly(A) (see, for example, Adesnik *et al.,* 1972). In addition, recent evidence shows that poly(A) shortens with time in the polyribosomes of Hela cells (Sheiness and Darnell, 1973).

We wish to characterize the mRNA of the simple eucaryote *Saccharomyces cerevisiae,* both with respect to its possible passage through precursor forms prior to entrance into polyribosomes, and with respect to its turnover in polyribosomes. This, we hope, will provide the background information necessary to begin to analyze mutants which are defective in RNA and protein synthesis. We report here the isolation and characterization of poly(A) containing RNA from yeast spheroplasts.

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Materials and Methods

(*a*) *Cell Growth and Preparation of Active Spheroplasts. Saccharomyces cerevisiae* (α s288c) was grown at 30° in minimal medium containing 7 g of Bacto yeast nitrogen base (without amino acids) and 20 g of glucose per liter.

Preparation of yeast spheroplasts was according to Hutchison and Hartwell (1967). Minimal medium, containing 0.4 M $MgSO_4$ as an osmotic stabilizer, was used to culture the spheroplasts. Our spheroplasts remain metabolically active for at least 4 hr in this medium and in this time interval synthesize bulk protein and RNA at the same rate as an identical concentration of intact cells.

RNA labeling was carried out with [^{3}H]adenine (specific activity 17 Ci/mmol) at 1.5 μCi/ml final concentration. Pulse labeling of spheroplasts for 3 min was done after a 2-hr incubation period. Labeling for 100 min, which began 20 min after resuspension of spheroplasts in culture medium, was carried out in the presence of 3.6 μg/ml of carrier adenine in addition to 1.5 μCi/ml of [^{3}H]adenine. This is a high enough adenine concentration to support linear uptake of [^{3}H]adenine over a 3-hr period.

(*b*) *Isolation of Total Spheroplast RNA.* Spheroplasts were harvested from culture by pouring over 0.5 vol of frozen, crushed 1 M sorbitol. They were collected by centrifugation (6000 rpm, 5 min, SS34 Sorvall rotor), resuspended in 0.2 vol of 1 M sorbitol, and again collected by centrifugation. The spheroplast pellet was dissolved in SDS[1] buffer (0.1 M NaCl–20 mM EDTA–20 mM Tris-HCl (pH 7.0)–0.5% SDS) and the RNA extracted using the SDS–hot phenol–chloroform method described by Penman (1969). We recover at least 80% of the RNA using this method.

(*c*) *Isolation of Polyribosomal RNA.* To minimize polyribosomal runoff during extraction, 100 μg/ml of cycloheximide was added to the spheroplasts at the end of the isotope incorporation period. Ten seconds later, spheroplasts were poured over a 0.5 culture volume of frozen, crushed 1 M sorbitol. Subsequent washing, lysis, and centrifugation were done at 4°.

Spheroplasts were collected by centrifugation (6000 rpm, 5 min, SS 34 Sorvall rotor), washed with 1 M sorbitol, and pelleted. They were then resuspended in the cell fractionation buffer of Udem and Warner (1973) (0.01 M NaCl–0.01 M Pipes–5 mM $MgCl_2$–1 mM dithiothreitol–0.01% spermidine HCl, adjusted to pH 6.5). After 5 min, 0.1 vol of 1% (w/v) saponin was added. The lysate was centrifuged at 10,000 rpm for 10 min to remove nuclei, mitochondria, and the few remaining unopened cells. The postmitochondrial supernatant was immediately layered over a 10–50% (w/v) linear sucrose gradient, prepared in cell fractionation buffer. Centrifugation was for 4 hr at 26,000 rpm at 4° in an SW 27 Beckman rotor.

The contents of the gradient were collected from the bottom of the tube through the flow cell of a recording spectrophotometer. Fractions of the gradient containing polyribosomes of two or more ribosomes were pooled and brought to 0.5% SDS. Ethanol (2 vol) was added to the pooled polyribosomes and they were stored at −20° overnight. Centrifugation at −20° for 1 hr at 10,000 rpm yielded a pellet containing all the RNA. This pellet was resuspended in SDS buffer and the RNA was extracted using the SDS–hot phenol–chloroform method.

(*d*) *Preparation and Use of Oligo(dT)-Cellulose.* Polymerization of TMP and attachment to cellulose in anhydrous pyridine were accomplished as described by Gilham (1964) and Edmonds (1972). Our oligo(dT)-cellulose hybridizes 2–4 A_{260} units of poly(A) per 100 mg of dry cellulose.

Standard fractionations by affinity chromatography were carried out at room temperature in columns (5-ml pipets) containing a bed volume of 1.5 ml of oligo(dT)-cellulose equilibrated with HS buffer (0.1 M NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.6), and 0.2% SDS). RNA from a maximum of 50 ml of spheroplasts was dissolved in 0.4 ml of HS buffer and layered on the column. The sample was allowed to flow into the column bed and held there for 30 min. The column was then washed with 15 ml of HS buffer to remove nonbound RNA species. Bound RNA was recovered by elution with 15 ml of LS buffer (10 mM Tris-HCl (pH 7.6)–0.2% SDS). The flow rates of our columns varied between 1 and 0.25 ml/min. Fractions of the high salt and low salt eluates containing radioactivity were pooled, brought to 0.1 M NaCl, and precipitated at −20° with 2 vol of ethanol. More than 95% of the input RNA is recovered from these columns. Recycling the RNA found in the high salt eluate and the low salt eluate through the binding and elution procedure was frequently used to ensure that the column capacity was not exceeded.

(*e*) *Electrophoretic Analysis of High Molecular Weight RNA.* RNA was subjected to electrophoresis in composite agarose–acrylamide gels. The gels (6 × 120 mm) contained 0.85% agarose (w/v) and 2% acrylamide (including 5% methylenebisacrylamide by weight) and were prepared and run in phosphate electrophoresis buffer (36 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA, and 0.2% SDS (pH 7.7)) according to Maurer (1971). Electrophoresis was started at 1 mA/gel for 15 min and then conducted at 5 mA/gel. Gels were sliced with a wire grid and consecutive slices, in groups of two, placed in vials containing 0.5 ml of 0.3 M KOH and held overnight at 45°. HCl (0.5 ml; 0.3 M) containing 50 mM Tris-HCl (pH 7.6) was added followed by 10 ml of scintillation fluid (1 l. of toluene, 4 g of 2,5-diphenyloxazole, 0.2 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, 500 ml of Triton X-100) and the isotope was determined by scintillation spectrometry. Recovery of [^{3}H]RNA from the gels was in excess of 80%.

(*f*) *Poly(A) Isolation.* Poly(adenylic acid) was released from large RNA molecules by virtue of its resistance in 0.3 M salt to the combined action of ribonucleases A and T_1 (Darnell *et al.*, 1971a). Nucleic acid, concentrated by ethanol precipitation, was resuspended in 0.5 ml of 0.3 M NaCl, 0.01 M Tris-HCl (pH 7.5), and 0.005 M $MgCl_2$ containing 50 μg of deoxyribonuclease 1 (Sigma type DN-ED). After incubation for 30 min at 37°, neutralized EDTA was added to a final concentration of 0.01 M. Ribonucleases A (Sigma type IIIA) and T_1 (Sigma type III) were added to final concentrations of 3 μg/ml and 5 units/ml, respectively. Incubation was continued for 60 min at 30°. RNA digestion was terminated by addition of 10 μg of preself-digested protease (Sigma type V) and sodium dodecyl sulfate to 0.2% followed by incubation for an additional 15 min. Poly(A) was separated from the other RNA digestion products by binding to oligo(dT)-cellulose.

(*g*) *Electrophoretic Analysis of Low Molecular Weight RNA.* Samples containing small RNA molecules were mixed with 80 μg of carrier tRNA and precipitated with 2 vol of ethanol at −20° overnight. The precipitate was recov-

[1] Abbreviations used are: SDS, sodium dodecyl sulfate; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

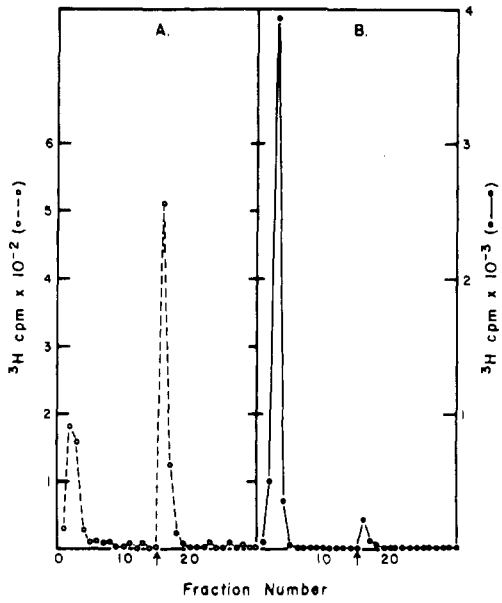

FIGURE 1: Fractionation of ^{3}H-labeled polyribosomal RNA by oligo(dT)-cellulose column chromatography. (A) Spheroplasts (50 ml) were labeled for 3 min with 75 μCi of [^{3}H]adenine (17 Ci/mmol). Polyribosomal RNA was extracted and fractionated as described under Materials and Methods. Isotope was measured in 10-μl samples of each 1-ml fraction after adding 100 μg of carrier RNA and precipitating with cold 5% Cl_3CCOOH. (B) Spheroplasts (50 ml) were labeled for 120 min with 75 μCi of [^{3}H]adenine (62 mCi/mmol). Polyribosomal RNA was extracted and fractionated as described under Materials and Methods. Isotope was measured in 20-μl samples of each 1-ml fraction as described above. The arrow (↑) designates when the LS buffer elution began.

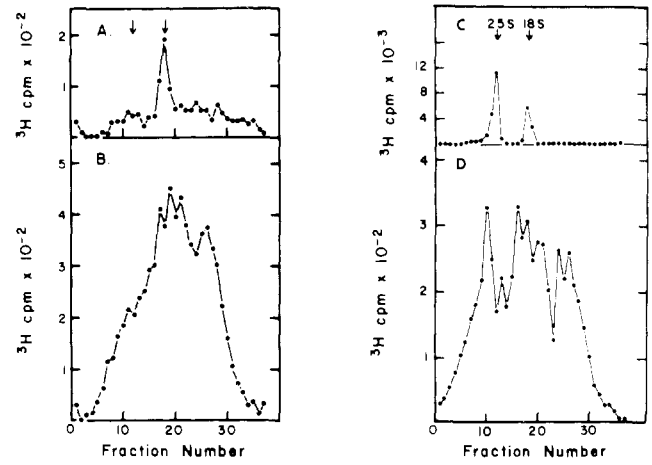

FIGURE 2: Electrophoresis of fractionated polyribosomal RNA in composite agarose-acrylamide gels. The RNA fractions obtained as described in Figure 1 were pooled, concentrated by ethanol precipitation, and subjected to electrophoresis at 5 mA/gel for 3 hr as described under Materials and Methods. Ribosomal RNA was run in a parallel gel to determine 25S and 18S marker positions: (A) RNA which does not bind to oligo(dT)-cellulose obtained from spheroplasts labeled for 3 min with [^{3}H]adenine; (B) poly(A) containing RNA obtained from spheroplasts labeled for 3 min with [^{3}H]adenine; (C) RNA which does not bind to oligo(dT)-cellulose obtained from spheroplasts labeled for 100 min with [^{3}H]adenine; (D) poly(A) containing RNA obtained from spheroplasts labeled for 100 min with [^{3}H]adenine.

ered by centrifugation (−20°, 10,000 rpm, 1 hr in the Sorvall SS 34 rotor) and dissolved in 0.1 ml of acetate electrophoresis buffer (20 mM sodium acetate–30 mM Tris–1 mM EDTA–0.2% SDS, pH adjusted to 7.8 with acetic acid). Glycerol (50%; 50 μl) and 3 μl of Bromophenol Blue were then added. The RNA was layered on a 6 × 100 mm gel containing 14% acrylamide, including 5% methylenebisacrylamide by weight, prepared as described by Maurer (1971). Electrophoresis was carried out for 3 hr at 5 mA/gel. Gels were sliced and processed for scintillation counting as described above.

(*h*) *Electrophoretic Analysis of RNA in 98% Formamide.* Purification of formamide and preparation of polyacrylamide gels were conducted essentially as described by Staynov *et al.* (1972) except that 1 M Tris-HCl (pH 7.6) was added to the purified formamide to a final concentration of 10 mM. High molecular weight RNA was analyzed in 4.2% (w/v) polyacrylamide gels containing 15% by weight *N,N'*-methylenebisacrylamide. Low molecular weight RNA was analyzed in 15% (w/v) polyacrylamide gels containing 10% by weight *N,N'*-methylenebisacrylamide. Gels were prerun for 15 min. RNA samples were resuspended in 50 μl of purified formamide and heated at 95° for 1 min. Then 3 μl of Bromophenol Blue and 40 μl of a solution containing 50% (w/v) sucrose (prepared in a 4:1 mixture of formamide and water) were added before layering onto the gel surface.

(*i*) *Preparation of Oligo(adenylic acid) Chains of Defined Length.* Commercial [^{14}C]poly(adenylic acid) (0.375 μCi) (Schwarz/Mann, 0.217 mCi/mmol of phosphate) was subjected to a limited 2-min hydrolysis in a 1-ml solution containing 0.015 M NaCl, 0.0015 M sodium citrate, and 176 μg of pancreatic ribonuclease A. The RNA chains in the digest were fractionated according to size by adsorption to and elution from an 0.9 × 5 cm DEAE-cellulose column as described by Heftman (1964).

(*j*) *Analysis of the Base Composition of RNA Samples.* Purines and pyrimidine 2',3'-monophosphates were prepared by acid hydrolysis of RNA samples and separated by chromatography on thin layers of cellulose as described by Leech *et al.* (1968). Hydrolysis products identified as fluorescent spots under an ultraviolet (uv) light were scraped into vials and isotope content determined by scintillation spectrometry as described above.

Results

Isolation and Characterization of Poly(A)-Containing RNA from Polyribosomes. Affinity chromatography on oligo(dT)-cellulose columns separates RNA molecules into two distinct fractions. Figure 1 shows two elution profiles of polyribosomal RNA from these columns. Sixty-four per cent of the labeled polyribosomal RNA from spheroplasts labeled with [^{3}H]adenine for 3 min (panel A) bound to oligo(dT)-cellulose. Only 7% of the labeled polyribosomal RNA bound when it was obtained from spheroplasts labeled continuously for 100 min (panel B).

Figure 2 shows an electrophoretic analysis in composite agarose-acrylamide gels of the fractions of RNA molecules shown in Figure 1. As expected ribosomal RNA does not bind to oligo(dT)-cellulose (panel C). In addition the same fraction of RNA obtained from pulse-labeled cells contains only a predominant peak of 18S rRNA (panel A). The observation that considerably less label is found in the 25S rRNA species after a short pulse confirms Udem and Warner's observation of the slower maturation and entrance of label into the larger ribosomal RNA in polyribosomes of yeast spheroplasts (Udem and Warner, 1972). The identity of the smaller molecules in this fraction which do not bind to oligo(dT)-cellulose remains to be specified. However, we know that none of this labeled RNA will bind to oligo(dT)-

TABLE I: Changes in the [^{14}C]Pyrimidine and [^{3}H]Purine Content of Yeast RNA during Poly(A) Purification.[a]

Steps in Purification	Total cpm in Cold 5% Cl_3CCOOH Precipitate		% of [^{3}H] in	
	^{14}C	^{3}H	A	G
RNA bound to 1st oligo(dT)-cellulose column [poly(A) containing RNA]	12,936	99,686	54	46
Nuclease digest	350	12,765		
RNA bound to 2nd oligo(dT)-cellulose column [poly(A)]	6	8,064	99	1

[a] Spheroplasts (50 ml) were divided into equal samples. One culture was exposed for 3 min to 1.5 μCi/ml of [^{3}H]adenine to label purines. The other culture was exposed to 3 min to 0.2 μCi/ml of [^{14}C]uracil to label pyrimidines. RNA was extracted from each culture as described under Materials and Methods; 286,700 cpm of [^{3}H]RNA and 41,900 cpm of [^{14}C]-RNA were mixed and the poly(A) sequences isolated according to procedures described under Materials and Methods. Isotope was measured in a sample of each fraction after addition of carrier RNA and precipitation with 5% cold Cl_3-CCOOH. A [^{14}C] overlap of 9% has been subtracted from the ^{3}H. ^{3}H overlap was negligible. [^{3}H]RNA remaining after the poly(A) purification steps and a sample of input [^{3}H]RNA were processed for purine composition as described under Materials and Methods.

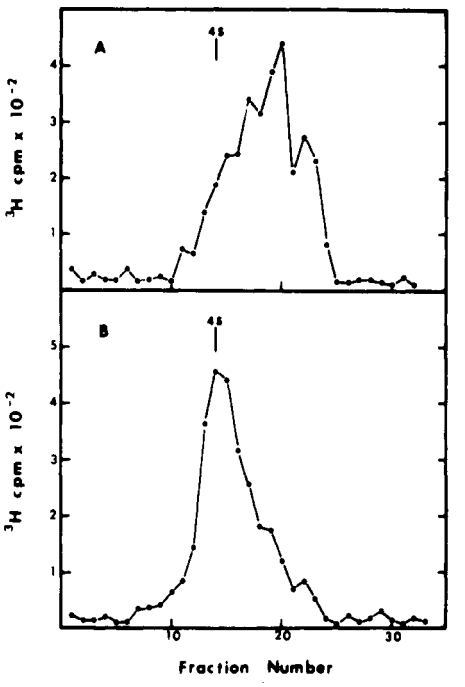

FIGURE 3: Electrophoresis in 14% polyacrylamide gels of poly(A) sequences from polyribosomal RNA. Poly(A) was obtained by nuclease digestion of polyribosomal poly(A) containing RNA and purified by oligo(dT)-cellulose chromatography as described under Materials and Methods. Carrier tRNA (80 μg) was added to the poly(A) example. The RNA was concentrated by ethanol precipitation and resuspended in HS buffer before application to the surface of the gel. Electrophoresis was for 3 hr at 5 mA/gel. 4S RNA standard was run in a parallel gel. The Bromophenol Blue dye migrated in fractions 20–22 in both gels: (A) poly(A) obtained from the polyribosomes of spheroplasts labeled for 100 min with [^{3}H]adenine; (B) poly(A) obtained from the polyribosomes of spheroplasts labeled for 3 min with [^{3}H]adenine.

cellulose when recycled through the affinity column (data not shown).

RNA species which bind to oligo(dT)-cellulose are shown in Figure 2, panels B and D. A comparison of the size patterns of molecules obtained by a 3-min or a 100-min labeling protocol shows a similarity between the two profiles especially in the region migrating faster than 25 S. An additional peak of RNA, however, is found in the 100-min labeled RNA profile, migrating slower than 25 S. This could represent RNA synthesized at a lower rate than the rest of the mRNA population and therefore not labeled during a short exposure to [^{3}H]adenine. The apparent weight average molecular weight of the molecules in Figure 2B,D is approximately 0.7×10^6. We assume that these are messenger RNA molecules because of their heterogeneous size distribution and because these polyribosomal RNA molecules are the most extensively labeled during a short pulse.

We obtain these large molecules from yeast only if precautions are taken to reduce cleavage of the RNA to a minimum during extraction and purification. (See Materials and Methods for the details of procedure.) The following results demonstrate that our techniques meet this condition. (1) Addition of heparin to 100 μg/ml before spheroplast lysis and in the sucrose gradients causes neither an increase in the fraction of labeled RNA which binds oligo(dT)-cellulose nor a change in the size distribution of this RNA in agarose–acrylamide gels. (2) We have recycled the pooled fractions of labeled RNA that bind to oligo(dT)-cellulose through the SDS–phenol–chloroform extraction, ethanol precipitation, and the affinity column. Greater than 90% of the counts still bind to oligo(dT)-cellulose.

Poly(A) Sequences in Messenger RNA. Poly(A) sequences were released from polyribosomal poly(A) containing RNA by the combined action of pancreatic ribonuclease A and ribonuclease T_1 (Darnell *et al.*, 1971a) and were separated from the other hydrolysis products by adsorption to oligo(dT)-celluloses as described under Materials and Methods.

The results presented in Table I confirm that this procedure leads to the isolation of poly(adenylic acid). RNA obtained from spheroplasts simultaneously labeled with [^{3}H]adenine and [^{14}C]uracil was subjected to the poly(A) isolation procedure. The amount of each isotope was followed through the purification and the composition of the remaining labeled RNA assessed by chromatography on thin layers of cellulose (Leech *et al.*, 1968).

Column two of Table I shows that essentially all of the ^{14}C-labeled isotope is removed from the RNA bound to the second oligo(dT)-cellulose column. Since the pyrimidine: purine ratio in yeast messenger RNA is about 0.8:1.0 (Kadowaki and Halvorson, 1971) a decline in the ^{14}C:^{3}H ratio during poly(A) purification to 0.6% of the value in poly(A) containing RNA indicates there is less than one pyrimidine residue per 100 purines in the final preparation. Analysis on thin-layer chromatography (tlc) plates of the composition of the remaining tritium labeled RNA demonstrates that 99% of the counts are in adenine (Table I, column 3) with the remainder found in guanine.

Figure 3A shows an electrophoretic analysis of poly(A) molecules obtained from messenger RNA labeled for 100 min. Since messenger RNA in these cells has an average

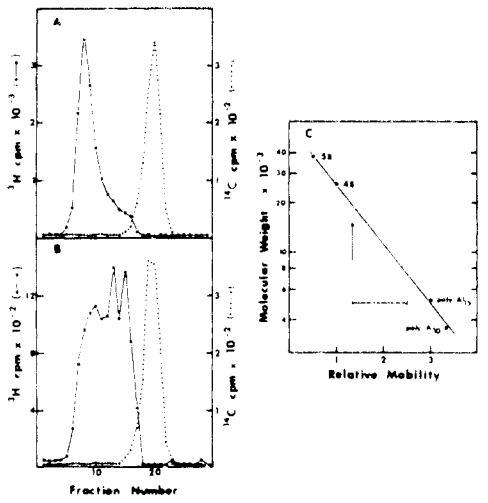

FIGURE 4: Molecular weight determination of poly(A) by electrophoresis in 15% polyacrylamide gels containing 98% formamide. Electrophoresis was for 4.5 hr at 5 mA/gel. A ^{14}C overlap of 18% has been subtracted from the ^{3}H: (A) poly(A) obtained from spheroplasts labeled for 3 min with [^{3}H]adenine (●—●), coelectrophoresed with a sample of [^{14}C]poly(A)$_{10}$ (● · · ●); (B) poly(A) obtained from spheroplasts labeled for 100 min with [^{3}H]adenine (●—●), coelectrophoresed with a sample of [^{14}C]poly(A)$_{10}$ (● · · ●); (C) standard molecular weight calibration curve obtained by an analysis of samples containing ^{3}H-labeled 5S and 4S RNAs and [^{14}C]poly(A)$_{15}$ or [^{14}C]poly(A)$_{10}$. The bracket (|— —|) indicates the region of the gel occupied by yeast poly(A) molecules. The arrow (↑) indicates the position occupied by the major peak of poly(A) found in pulse labeled spheroplasts.

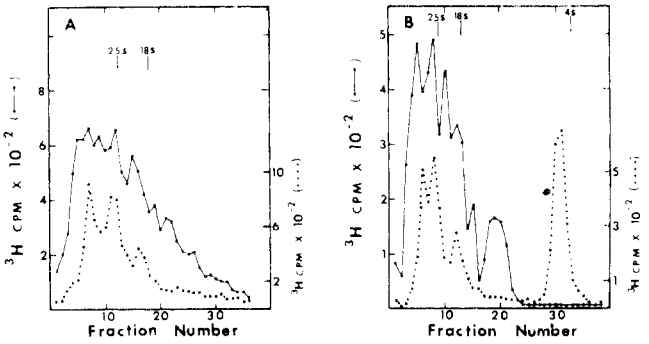

FIGURE 5: Electrophoretic analysis of total cellular RNA obtained from spheroplasts labeled for 3 min with [^{3}H]adenine. Spheroplasts (50 ml) were labeled for 3 min with [^{3}H]adenine. Total RNA was extracted and fractionated on oligo(dT)-cellulose as described under Materials and Methods. A portion of each sample was used for electrophoretic analysis of the RNA. Each panel contains superimposed gel profiles of poly(A) containing RNA (●—●) and RNA which does not bind to oligo(dT)-cellulose (● · · ●); 86 and 92% of the input labeled poly(A) containing RNA was recovered in the gels in panels A and B, respectively. (A) Electrophoretic analysis in 2% acrylamide 0.85% agarose gels prepared in aqueous buffer. Electrophoresis was carried out at 5 mA/gel for 3 hr. 4S RNA has just run off the end of the gels. (B) Electrophoretic analysis in 4.2% acrylamide gels prepared in 98% formamide. Electrophoresis was carried out at 5 mA/gel for 3 hr and 20 min.

half-life of about 20 min (Hutchison *et al.*, 1969; N. Hynes, unpublished results), a 100-min continuous labeling will fully label the messenger RNA pool. Thus, the poly(A) size distribution seen in Figure 3A closely approaches an authentic mass distribution of these molecules. A broad asymmetric peak of poly(A) spread from 4 S to the Bromophenol Blue dye marker is apparent in the gel.

If poly(A) length in a messenger RNA molecule is an indicator of the age of this molecule or the rate this molecule turns over, one might expect an analysis of poly(A) in pulse labeled messenger RNA to reveal a larger and perhaps a more homogeneous population of molecules. This is demonstrated in Figure 3B. The distribution of poly(A) obtained from polysomes labeled for 3 min has a predominant peak of molecules comigrating with 4S RNA. Although the size distribution of the poly(A) populations labeled for 3 and 100 min is different, poly(A) of large as well as small size is present in both cases. A comparison between the two poly(A) populations shows that poly(A) labeled for 3 min is of higher average molecular weight and less heterogeneous in size. The size distribution in Figure 3B is similar to that reported by McLaughlin *et al.* (1973) and confirms that these species of poly(A) are considerably shorter than the poly(A) regions in mammalian messenger RNA.

A Length Calibration of Poly(A) Chains. To precisely define the length of the poly(A) sequences, electrophoretic analysis in 15% polyacrylamide gels prepared in 98% formamide was carried out according to Staynov *et al.* (1972). Under these conditions inter- and intramolecular secondary structure in RNA chains is minimized. 4S and 5S RNAs and smaller [^{14}C]oligo(adenylic acid) chains of defined length were used as standards. Figure 4, panels A and B, presents an analysis of poly(A) obtained from the polyribosomes of spheroplasts labeled with [^{3}H]adenine for 3 and 100 min, respectively. [^{14}C]Oligo(A)$_{10}$ was included in each sample to ensure correct alignment of the two gels. The results confirm the observations presented in Figure 3 and rule out the possibility that the large poly(A) chains seen in RNA labeled during a pulse are the consequence of aggregation. From the standard curve presented in Figure 4C, it can be seen that polyribosomal poly(A) chains have molecular weights ranging from 7500 to 20,000 (about 20–60 nucleotides). The range in molecular size is indicated by the bracket in Figure 4C. Poly(A) sequences obtained from the RNA molecules labeled during a pulse are approximately 60 nucleotides in length (↑).

Large, Rapidly Labeled Poly(A) Containing RNA in Total Cell Extracts. Characterization of total cellular RNA from pulse labeled spheroplasts may provide information about nuclear RNA species, since only about 20% of the RNA made during a 3-min pulse is found in cytoplasmic polyribosomes. Additionally, the length of poly(A) containing RNA should be minimally effected by nuclease degradation in spheroplasts harvested directly into SDS buffer. Thirty-six per cent of the labeled RNA obtained by direct extraction of spheroplasts labeled with [^{3}H]adenine for 3 min binds to oligo(dT)-cellulose. The poly(A) sequences in the RNA obtained from pulse labeled spheroplasts are the same size as the poly(A) sequences found in pulse labeled messenger RNA (data not shown). Figure 5A shows an electrophoretic analysis in composite agarose–acrylamide gels of the RNA fractions obtained from an oligo(dT)-cellulose fractionation. The precursor ribosomal RNAs do not bind to the column. This has been confirmed using [^{3}H]methyl-labeled precursor ribosomal RNA. The poly(A) containing RNA has a broad size distribution with a large fraction of the molecules migrating more slowly than 25S rRNA. This distribution is distinctly larger than the messenger RNA size distribution (compare Figures 2B and 5A). To determine whether the very large poly(A) containing RNAs are multimolecular aggregates or whether they are covalently intact structures we conducted an electrophoretic analysis in 4.2% polyacrylamide gels prepared in 98% formamide. Figure 5B presents this analysis of the same RNA preparations exhibited in Figure 5A. Again much of the poly(A) containing RNA migrates more slowly

than 25S ribosomal RNA. Some species are as large as the 35S precursor ribosomal RNA (2.4×10^6).

Discussion

Poly(A) Size and Messenger RNA Turnover. The change in size distribution of poly(A) with labeling time seems to be a unique property of the population of poly(A) molecules and not the intact mRNA population, for the size distribution of pulse labeled and long term labeled poly(A) containing RNA is similar (compare Figures 2B and 2D). Thus, [^{3}H]adenine first appears in a broad size range of mRNAs which all contain longer species of poly(A).

These data can be accounted for by two different models which describe the relationship between poly(A) content and the turnover of mRNA. Either those mRNA molecules which contain the longest poly(A) sequences have the shortest lag time between synthesis and appearance in the polyribosomes or, as an mRNA molecule functions in the polyribosomes, its poly(A) region gets shorter with time. At this time we are unable to discriminate between these alternatives in yeast. An assessment of whether the source of the size heterogeneity is nuclear or cytoplasmic will perhaps permit a choice between these two models. However, in HeLa cells, it has been demonstrated that poly(A) does seem to shorten with time in the polyribosomes (Sheiness and Darnell, 1973). Thus, serious consideration must be given to the possibility that, in yeast, poly(A) length may shorten as messenger RNA functions in polyribosomes.

The Number of mRNAs Containing Different Sizes of Poly(A). Electrophoretic analysis of uniformly labeled RNA molecules has enabled us to look at the mass distribution of poly(A) as a function of size. Clearly, a more informative parameter is the number distribution among poly(A) lengths, for it is of interest to know the relative numbers of mRNA molecules which contain long and short poly(A). Correction across the poly(A) mass distribution in Figure 5B for changes in molecular weight enables us to estimate the relative number distribution with respect to size. Thus we estimate that less than 10% of all mRNA molecules isolated on oligo(dT)-cellulose carry poly(A) chains greater than 55 nucleotides in length. Fifty per cent of all mRNA molecules isolated on oligo(dT)-cellulose carry poly(A) chains less than 32 nucleotides in length.

However, a test of the sensitivity of our techniques for detecting very short poly(A) sequences reveals that $poly(A)_{15}$ does not bind to oligo(dT)-cellulose! Thus, it is possible that messenger RNA molecules carry even shorter poly(A) sequences. The pulse labeled RNA migrating faster than 18S rRNA in Figure 2A might represent such a population of molecules.

Large Poly(A) Containing RNA in Total RNA Extracts of Spheroplasts. The largest RNA molecules found in mammalian cells belong to a class of molecules known as heterogeneous nuclear RNA. Many of these molecules turn over very rapidly, contain poly(A), and are considerably larger than 4×10^6 daltons in size. Heterogeneous nuclear RNA is thought to contain messenger precursor RNA (see Darnell *et al.* (1973) for a review). Precursors to ribosomal and messenger RNA are likely to be specifically labeled if spheroplasts are exposed to a short enough pulse of [^{3}H]adenine. The experiments presented here demonstrate that this condition is met for the precursors to ribosomal RNA (Figure 5). However, our results fail to reveal a population of poly(A) containing RNA molecules in yeast which are as large as the giant heterogeneous nucleus RNA molecule in mammalian cells. The largest poly(A) containing RNA species obtained from 3-min pulse labeled spheroplasts harvested directly into SDS buffer are approximately 2.4×10^6 daltons in size (Figure 5). These results are more in agreement with observations in the cellular slime mold, *Dictyostelium discoideum,* where Firtel and Lodish (1973) demonstrated that nuclear poly(A) containing RNA is only about 20% larger than polyribosomal poly(A) containing RNA. However, we cannot exclude the possibility that a small number of larger species of poly(A) containing RNA may be obscured under the trailing shoulder of the largest RNA species in Figure 5. Our data do suggest that precursors to some mRNA molecules may be cleaved to a smaller size before incorporation into polyribosomes. Our current studies of yeast nuclei should resolve this point and provide important information about messenger precursor RNA in yeast.

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