chromatin template capacity (O'Malley *et al.*, 1969, 1971), and RNA polymerase activity (McGuire and O'Malley, 1968). These alterations are paralleled by appearance of new species of hybridizable nuclear RNA (O'Malley and McGuire, 1968b,c, 1969). A conversion of ribosomes to polyribosomes then occurs, presumably due to the entry of new messenger RNA into the cytoplasm. These polyribosomes synthesize a qualitatively different population of peptides *in vitro* which presumably reflect earlier alterations in messenger RNA. Finally, the end product of this action of progesterone is the induction *de novo* of avidin synthesis. This sequence of events indicates, therefore, that progesterone exerts its primary effect on the nucleus to promote selective gene activation and subsequent synthesis of new avidin molecules. Studies designed to identify avidin molecules synthesized *in vitro* by oviduct polyribosomes are now in progress.

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Properties of a Polyriboadenylate Polymerase Isolated from Yeast Ribosomes*

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ABSTRACT: An enzyme isolated from yeast ribosomes catalyzes a primer-dependent synthesis of short polyriboadenylate chains from ATP. Other ribonucleoside triphosphates (UTP, GTP, and CTP) are not substrates, but when present individually or together with ATP result in inhibition of AMP polymerization. The reaction requires Mn^{2+} (10^{-3} M) or Mg^{2+} (10^{-2} M) for optimal activity. Yeast ribosomal RNA is a better primer than synthetic polyribonucleotides; yeast transfer RNA and calf thymus DNA (native or denatured) are inactive. Evidence is presented for the covalent linkage of the polyriboadenylate to the 3-hydroxyl end of the primer. The chain length of the polyriboadenylate (10–20 AMP residues) is dependent on time of incubation and primer RNA concentration.

Enzymes which catalyze the terminal polymerization of adenylate residues from ATP to the 3-hydroxyl end of polyribonucleotides have been detected in mammalian tissues (Edmonds and Abrams, 1960; Klemperer, 1963; Burdon, 1963), chick embryos (Venkataraman and Mahler, 1963), sea urchin embryos (Hyatt, 1967), and bacteria (August *et al.*, 1962; Gottesman *et al.*, 1962; Payne and Boezi, 1970). Although a physiological function for this type of polymerization reaction has not yet been defined, the isolation of adenylate-rich polynucleotides from rat liver (Hadjivassiliou and Brawerman, 1966), Ehrlich ascites cells (Edmonds and Cara-

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mela, 1969), and virus (Bellamy and Joklik, 1967) suggests that the observed *in vitro* reaction is not just an artifact of the isolated enzyme. The apparent polymerization of adenylate residues from ATP by yeast cell-free extracts was noted in earlier work concerned with protein biosynthesis (Bretthauer *et al.*, 1963). This report is concerned with the isolation and properties of the ribosomal-bound enzyme from yeast which catalyzes the RNA primer-dependent synthesis of oligoadenylates from ATP.

Materials and Methods

Materials. [8-^{14}C]ATP, [^{3}H]GTP, [^{3}H]dATP, [^{32}P]inorganic phosphate, and polylysine were purchased from New England Nuclear. [8-^{14}C]ADP, [2-^{14}C]CTP, and [2-^{14}C]UTP were obtained from Schwarz. Other unlabeled nucleoside di- and triphosphates and highly polymerized calf thymus DNA were from Sigma. Poly(A) and poly(U) were from Miles Laboratories. Bovine pancreatic ribonuclease (5× crystallized) was purchased from Calbiochem. Sephadex was obtained from Pharmacia and DEAE-cellulose from Bio-Rad. Yeast nuclease II (endonuclease yielding 5′-phosphate esters) was prepared according to published procedures (Nakao *et al.*, 1968; Lee *et al.*, 1968). Yeast ribosomal RNA was extracted from washed ribosomes with 2 M LiCl (Curry and Hersh, 1962) and dialyzed extensively against 0.05 M Tris-HCl buffer, pH 8.5, before use.

Enzyme Assays. The poly(A) polymerase assay contained, unless otherwise indicated, the following components in 0.5-ml total volume: 0.5 mM [^{14}C]ATP (0.2 μCi/μmole); 40 mM Tris-HCl buffer, pH 8.5; 4 mM mercaptoethanol; 1 mM $MnCl_2$; 40 A_{260} units of ribosomal RNA; and enzyme. After incubation at 30° for various times, aliquots (usually 0.1 ml) were withdrawn and applied to a 2-cm diameter disk of Whatman No. 3MM paper. The paper disks were partially dried with a stream of warm air and immersed in cold 5% trichloroacetic acid containing 0.01 M $Na_4P_2O_7$. After 15 min, the disks were washed successively with: 5% trichloroacetic acid, twice; ethanol–ether (1:1, v/v); ether. The dried disks were then counted in a liquid scintillation counter. Enzyme assays terminated at zero time with 5% trichloroacetic acid were routinely run and any radioactivity remaining on the paper disk was substracted from other assays. The results obtained with this paper disk assay agreed very well with results obtained by collecting and washing the trichloroacetic acid precipitates by centrifugation and counting the Hyamine hydroxide solubilized product. One unit of enzyme is defined as that amount in the 0.5-ml assay mixture which catalyzes the incorporation of 1 nmole of AMP into trichloroacetic acid insoluble material in 30 min at 30°.

The assay for DNA-dependent RNA polymerase was carried out exactly as described by Frederick *et al.* (1969) using [^{14}C]ATP as the labeled ribonucleoside triphosphate and either native or alkali-denatured calf thymus DNA. The paper disk assay as previously described was used to determine any [^{14}C]AMP incorporation into product.

Polynucleotide phosphorylase was assayed by the formation of [^{32}P]ADP from poly(A) and [^{32}P]P_i, using either charcoal adsorption or paper chromatography for identification of [^{32}P]ADP (Singer, 1966).

The reaction mixture for the assay of ribonuclease contained in 0.5 ml: 40 mM Tris-HCl buffer, pH 8.5; 4 mM mercaptoethanol; 1 mM $MnCl_2$; 0.4% RNA; and enzyme. After incubation at 30° for various times, 0.1-ml aliquots were mixed with 0.2 ml of 0.25% uranyl acetate in 2.5% perchloric acid and allowed to sit in an ice bath for 15 min. Following centrifugation, the absorption at 260 mμ was determined on the supernatant fluid. One unit of ribonuclease is that amount in the 0.5-ml assay mixture which produces 1 A_{260} of acid-soluble nucleotides in 2 hr at 30°.

Measurement of PP_i. Standard enzyme assay mixtures were scaled up to 1.5 ml and incubated for 30 min. An aliquot (0.1 ml) was removed for determination of [^{14}C]AMP incorporation into acid-insoluble product. Another aliquot (1.0 ml) was acidified and charcoal added as described by August *et al.* (1962). The amount of P_i was measured before and after acid hydrolysis (in 1 N H_2SO_4, 100°, 7 min), the difference being proportional to PP_i.

Chain-Length Determination. To the reaction mixtures was added 3 ml of cold 5% trichloroacetic acid, and the precipitated protein and nucleic acid were isolated and washed twice by centrifugation with 3 ml of 5% trichloroacetic acid. The precipitate was dissolved in 0.2 ml of 0.3 M KOH and incubated for 18 hr at 37°. The pH was adjusted to 4.5 with 10% perchloric acid, and the potassium perchlorate was removed and washed once with 0.1 ml of water by centrifugation. Decending chromatography of the hydrolysate was carried out on Whatman No. 3MM paper with the solvent isobutyric acid–ammonium hydroxide–water (66:1:33, v/v). After locating the 2′(3′)-AMP and adenosine with the Packard radiochromatogram scanner, the radioactive areas were cut into strips and counted in a liquid scintillation counter.

Preparation of Oligoribonucleotides. Oligonucleotides bearing 3′-hydroxyl end groups were prepared with yeast nuclease in an incubation mixture containing the following components in 0.5 ml; 20 mM Tris-HCl buffer, pH 7.6; 10 mM $MgCl_2$; either 1.1 mg of poly(U) or 26 A_{260} units of yeast RNA; 0.1 ml of yeast nuclease. Aliquots (0.1 ml) were withdrawn after 0-, 10-, 20-, and 30-min incubation at 37° and heated in a boiling-water bath for 2 min to denature the nuclease. These 0.1-ml aliquots were then used as primers in the poly(A) polymerase assay.

Oligonucleotides with 3′-phosphate end groups were prepared by pancreatic ribonuclease digestion of poly(U) or yeast RNA. Reaction mixtures contained in 0.5 ml: 14 mM Tris-HCl buffer, pH 7.6; 26 A_{260} units of yeast RNA or 1.4 mg of poly(U); 2 μg of crystalline pancreatic ribonuclease. The individual 0.1-ml aliquots removed after 0-, 10-, 20-, and 30-min incubation at 37° were immediately applied to a 1 × 3 cm column of Bio-Rex 70 cation-exchange resin to remove the ribonuclease. Elution with H_2O was carried out until no further absorption at 260 mμ of the eluate was detectable. The eluates were lyophylized and redissolved in 0.1 ml of 0.02 M Tris-HCl buffer, pH 8.5, and used as primers.

With both the yeast nuclease and pancreatic ribonuclease, preliminary experiments were carried out to determine that after 30-min incubation, approximately 50% of the RNA or poly(U) was converted into 5% trichloroacetic acid soluble oligonucleotides.

Other Methods. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard, P_i by the method of Fiske and Subbarow (1925), and PP_i as P_i after hydrolysis in 1 N H_2SO_4 at 100° for 7 min.

Results

Distribution of Enzyme. Poly(A) polymerase activity was originally detected in the ribosome fraction. A study of the distribution of the enzyme in ribosomes and ribosome supernatant fluid revealed that the enzyme was also present in the

TABLE I: Cellular Distribution of Poly(A) Polymerase at Different Stages of Growth.[a]

Growth Time (hr)	Absorbance of Medium (600 mμ)	Total Units[b]		Specific Activity (Units/mg)	
		Sup	Rib.	Sup	Rib.
5	4.0	150	512	2.9	14.3
8	7.1	167	415	3.2	8.7
10	9.2	191	378	3.3	5.2

[a] A 20-ml aliquot was withdrawn from a growing culture at the times indicated. Ribosomal and supernatant fractions were prepared as described in Enzyme Isolation. [b] Units of enzyme are minimal due to rapid degradation of the ^{14}C product by nucleases.

soluble supernatant fraction. However, the ratio of total enzyme activity and specific activity in these 2 fractions varied with time of growth of the yeast (Table I). Some 75% of the total enzyme was found in the ribosomal fraction during early logarithmic growth, this enzyme having a specific activity 4–5 times higher than the supernatant enzyme. Therefore, for purification purposes, cells were harvested during early logarithmic growth and only the ribosomal fraction was used.

Enzyme Isolation. The hybrid yeast *Saccharomyces fragilis* X *S. dobzhanskii* was grown as described by Bretthauer *et al.* (1963) and harvested by centrifugation when the absorbance of the culture at 600 mμ was 3.7. Typically, cells from 30 l. of growth medium were washed with cold 0.05 M Tris-HCl buffer, pH 7.6, and disrupted with a French pressure cell at 6000–9000 psi. All of the following steps were carried out at 4°. The cell extract was centrifuged at 5000 rpm in the Sorvall SS-34 rotor for 5 min. The resulting supernatant fluid was centrifuged at 15,000 rpm and 20,000 rpm for 20 and 30 min, respectively. The clear supernatant fluid (230 ml), referred to as the crude extract, was then centrifuged at 50,000 rpm in the Spinco Type 50 rotor for 2 hr. The ribosomal pellet was rinsed with 0.05 M Tris-HCl buffer, pH 7.6, and suspended in the same buffer to a final volume of 98 ml.

The ribosome suspension (30 ml) was mixed with 30 ml of a solution containing 1.0 M NH_4Cl, 0.2 M $MgCl_2$, 0.5 M sucrose, and 0.06 M Tris-HCl buffer, pH 7.6, and allowed to stand in an ice bath for 30 min. This mixture (20 ml) was then layered on top of 10 ml of a solution containing 0.5 M NH_4Cl, 0.1 M $MgCl_2$, 2 M sucrose, and 0.03 M Tris-HCl buffer, pH 7.6, in each of three Spinco Type SW-25 rotor centrifuge tubes (Bruening and Bock, 1967). After centrifugation at 25,000 rpm for 12 hr, three distinct layers were obtained. The top 22 ml of each tube was carefully pipetted off and dialyzed against 0.05 M Tris-HCl buffer, pH 7.6. A total of 213 ml was obtained from the 98 ml of original ribosome suspension. This solution is referred to as the high-salt wash.

TABLE II: Summary of Purification of Poly(A) Polymerase from Ribosomes.

Fraction	Total Protein (mg)	Total Units	Specific Activity (Units/mg)	Total RNase Units
Crude Extract	4671	9827	2.1	14835
Ribosomes	1534	3185	2.0	5365
High-salt wash	226	9495	42	1115
$(NH_4)_2SO_4$	81	11528	142	0

TABLE III: Requirements for Optimal Enzyme Activity.

Omission	AMP Incorporated (nmoles/30 min)	% of Complete
None	107.9	100
Mn^{2+}	0.4	0.4
Mercaptoethanol	31.7	29.4
Yeast RNA	12.4	11.5

To the 213 ml of the high-salt wash solution was added with stirring 53 g of $(NH_4)_2SO_4$, and after 10 min, the precipitate was removed by centrifugation and discarded. An additional 28 g of $(NH_4)_2SO_4$ was added to the supernatant fluid. The precipitate obtained was dissolved in 92 ml of 0.05 M Tris-HCl buffer, pH 7.6, and dialyzed against the same buffer.

Attempts to further purify the enzyme by chromatography on DEAE-cellulose or CM-cellulose did not result in any increase in specific activity, although some nucleic acid was removed on DEAE-cellulose. As enzyme activity was also lost, this step was not routinely used.

A summary of the purification achieved is given in Table II. The increase in total units obtained in the high-salt wash fraction may be due to latency of the enzyme while attached to the ribosomes, or, more likely, due to the fact that only some 20% of the ribosomal-associated ribonuclease is removed by this procedure. The nuclease present in the ribosomal fraction would result in an underestimation of poly(A) polymerase activity due to degradation of the synthesized product.

Contaminating Enzymes. When assayed as described in Methods with protein concentrations used in the poly(A) polymerase assay, the $(NH_4)_2SO_4$-fractionated enzyme exhibited no ribonuclease activity (see Table II) or DNA-dependent RNA polymerase activity.

With [^{14}C]ADP as substrate, some radioactivity was incorporated into 5% trichloroacetic acid insoluble product, suggesting polynucleotide phosphorylase activity. However, when poly(A) and [^{32}P]P_i were incubated with the enzyme, no detectable [^{32}P]ADP or other ^{32}P-containing nucleotides were formed. In addition, no effect on activity with either [^{14}C]ADP or [^{14}C]ATP was noted upon addition of other electrolytes such as KCl, NaCl, or poly(lysine) which have been shown to simulate poly(A) synthesis by *Clostridium perfringens* polynucleotide phosphorylase (Fitt *et al.*, 1968). The incorporation of radioactivity from [^{14}C]ADP is apparently due to the formation of [^{14}C]ATP by contaminating adenylate kinase. The time-dependent synthesis of both [^{14}C]ATP and [^{14}C]AMP could be demonstrated when enzyme was incubated with [^{14}C]ADP in the absence of primer RNA. After 15-min incubation, 8, 18, and 16% of the initial [^{14}C]-ADP was recovered as [^{14}C]ATP, [^{14}C]AMP, and [^{14}C]adenosine, respectively [by paper chromatography in isobutyric acid–NH_4OH–H_2O (66:1:33, v/v) and counting of the paper

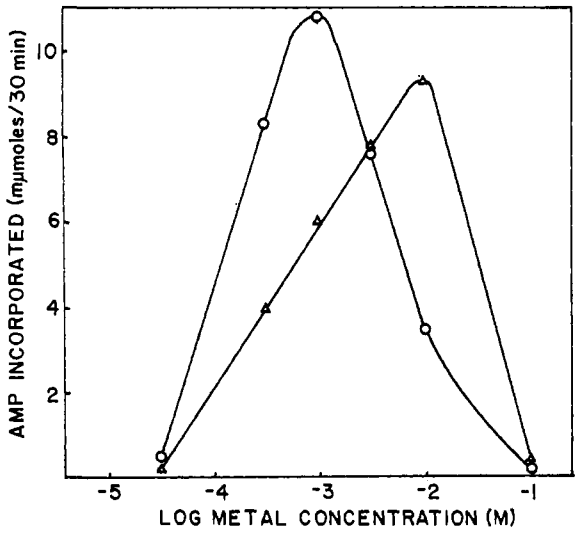

FIGURE 1: Dependence of reaction on concentration of Mn^{2+} or Mg^{2+}. The standard assay as described in Materials and Methods was used except the concentration of either Mn^{2+} (○—○) or Mg^{2+} (△—△) was varied.

strips]. The [^{14}C]adenosine is presumably formed from the presence of contaminating phosphatase activity. Inorganic pyrophosphatase activity was not detected, thus allowing the measurement of stoichiometric amounts of PP_i formed in the polymerase reaction with [^{14}C]ATP as substrate.

Requirements of Reaction. At the optimal pH of 8.5 and with components in the assay mixture as described in Methods, the reaction was proportional to enzyme concentration and time, up to at least 30 min.

All of the components in the assay mixture are required, omission of any one component (Mn^{2+}, mercaptoethanol, or RNA) resulting in a marked decrease in activity (Table III).

The reaction is dependent on the presence of either Mn^{2+} or Mg^{2+}, 10^{-3} M or 10^{-2} M concentration being optimal, respectively (Figure 1). Inhibition of the reaction was noted upon addition of Mg^{2+} to mixtures containing 10^{-3} M Mn^{2+}.

Dependence of the reaction on yeast ribosomal RNA concentration is shown in Figure 2. Near saturation was attained at 40 A_{260} units/0.5 ml of reaction mixture. Some incorporation of AMP into product was always noted without added primer. That this was due to nucleic acid contamination of the enzyme preparation was indicated by the A_{260}/A_{280} ratio of 1.3.

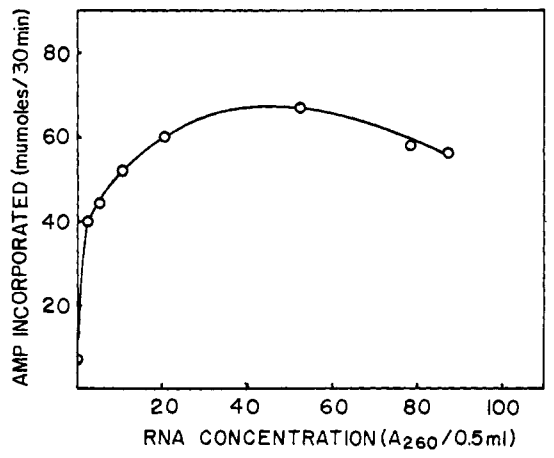

FIGURE 2: Dependence of the reaction on RNA concentration. The standard enzyme assay was used as described in Materials and Methods. The concentration of yeast ribosomal RNA was varied as indicated.

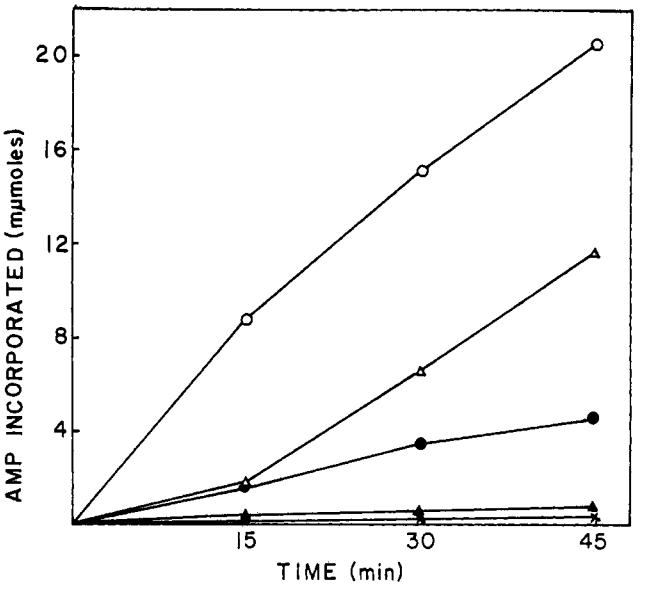

FIGURE 3: Effect of various polynucleotides on enzyme activity. The standard 0.5-ml assay mixture contained either 25 A_{260} units of yeast ribosomal RNA (○—○), 1.2 mg of poly(A) (△—△), 1.2 mg of poly(U) (●—●), 1.2 mg of yeast transfer RNA (▲—▲), or 0.75 mg of native or denatured calf thymus DNA (×—×).

Kinetic studies with various synthetic polyribonucleotides, native or denatured DNA, and transfer RNA as primers showed that the latter two nucleic acids were essentially inactive, whereas poly(A) and poly(U) were active but led to slower rates of enzyme activity than with ribosomal RNA (Figure 3).

Substrate Specificity. Of the 4 common ribonucleoside triphosphates, only ATP was active with the enzyme (the other 3 incorporated less than 1% of ATP) (Table IV). The K_m for ATP as determined from a Lineweaver–Burk plot was 4.5×10^{-4} M. The rate of incorporation of dAMP from dATP was 6.5% that of ATP. The enzyme preparation also catalyzed the incorporation of AMP from ADP to the extent of 33% that of ATP. As discussed previously, this is apparently due to the presence of contaminating adenylate kinase.

TABLE IV: Substrate Specificity of Yeast Poly(A) Polymerase.

Substrate[a]	Nucleotide Incorporated (nmoles/30 min)	Activity (% of ATP)
[^{14}C]ATP	107.9	100
[^{14}C]UTP	0.7	0.6
[^{14}C]CTP	0.8	0.7
[^{3}H]GTP	0.3	0.3
[^{3}H]dATP	7.0	6.5
[^{14}C]ADP	35.2	32.7

[a] All substrates were present at 0.5 mM concentration. The specific activity for all ^{14}C substrates was 0.2 μCi/μmole and for ^{3}H substrates was 0.4 μCi/μmole.

TABLE V: Inhibition of Enzyme Activity with ATP by Other Nucleotides.

Additions[a]	AMP Incorporated (nmoles/30 min)	Inhibn (%)
None	107.9	0
UTP	84.7	21.4
CTP	53.4	51.5
GTP	46.5	56.8
UTP, CTP, GTP	26.3	75.6

[a] All nucleotides added to the standard assay mixture were present at 0.5 mM concentration each.

Inhibition by Other Nucleotides. The other ribonucleoside triphosphates, CTP, UTP, and GTP, each inhibited the enzymatic polymerization of AMP from ATP with GTP being the best inhibitor and UTP the poorest (Table V). The reaction was inhibited 75% with all 3 of these nucleotides present, but was not completely additive.

Formation of PP_i. Reaction mixtures were analyzed both for [^{14}C]AMP incorporation into 5% trichloroacetic acid insoluble products and for PP_i formation as described in Methods. After 30-min incubation, 49 nmoles of [^{14}C]AMP was incorporated into product and 47 nmoles of PP_i was formed. Control incubations without added primer RNA or enzyme revealed negligible quantities of PP_i formed, although P_i was formed from the phosphatases present.

Chain Length of Product. The amount of radioactivity in 2′(3′)-AMP and adenosine recovered from alkaline hydrolysates of reaction mixtures was determined and used as an estimate of the average number of adenylate residues polymerized in a given chain. In Table VI are shown results with different primer RNA concentrations and with different times of incubation. Increasing the concentration of RNA from 19 to 57 A_{260} units per 0.5 ml of incubation mixture resulted in the progressive shortening of the average chain length of the product (Table VI, expt 1). This is indicated by the ratio of radioactivity recovered in 2′(3′)-AMP and adenosine to that of adenosine decreasing from 18 to 9. Such results are expected for a primer-dependent reaction, as more RNA molecules would lead to the initiation of more chains which would be shorter at any given time. With a fixed concentration of RNA (38 A_{260} units/0.5 ml), the chain length increases slightly with increasing times of incubation (Table VI, expt 2). Both experiments indicate that only relatively few (10–20) adenylate residues are added to the RNA primer molecules.

TABLE VI: Chain Length of Product with Different RNA Concentrations and Incubation Times.[a]

Concentration of RNA (A_{260} Units/0.5 ml)	Incubation Time (min)	Radioactivity (cpm/0.5 ml) 2′(3′)-AMP	Radioactivity (cpm/0.5 ml) Adenosine	Chain Length
Expt 1				
19	60	8526	509	18
38	60	7477	680	12
57	60	6535	843	9
Expt 2				
38	10	1540	146	12
38	20	3047	251	13
38	30	4258	289	15

[a] The standard assay was used with concentrations of RNA and times of incubation as indicated. Alkaline hydrolysis and chromatography are described in Materials and Methods.

TABLE VII: Effect of Nuclease Digestion on Primer Activity of Poly(U) and Ribosomal RNA.[a]

Nuclease	Digestion Time (min)	% Activity with RNA	% Activity with Poly(U)
Pancreatic	0	100	100
	10	85	105
	20	70	84
	30	38	60
Yeast	0	100	100
	10	107	151
	20	129	196
	30	139	210

[a] RNA and poly(U) were digested with nuclease as described in Materials and Methods. The 0.1-ml aliquots of nuclease-digested nucleic acids were added to the standard assay mixture (0.15-ml total volume) and incubated at 30° for 30 min. Reactions were stopped by placing the tubes in a boiling H_2O bath for 2 min. Samples were spotted on Whatman No. 3MM paper and irrigated with isobutyric acid–NH_4OH–H_2O (66:1:33, v/v). Radioactivity remaining at the origin was determined by counting of strips in the liquid scintillation counter.

Association of Product with RNA Primer. Evidence for the covalent linkage of the oligoadenylate to the RNA primer was obtained by sucrose gradient centrifugation and Sephadex gel filtration of reaction mixtures, and by modification of the 5′ or 3′ ends of the primer RNA. The former experiments were carried out by centrifuging redissolved trichloroacetic acid precipitates of reaction mixtures on 5–20% sucrose gradients as described in Figure 4. After 30-min incubation, the ^{14}C product was found to sediment at the same rate as the primer RNA and is therefore apparently associated with all RNA species present. No radioactivity was present in a zero-time incubation control, thus eliminating the possibility that any of the radioactivity found in the 30-min incubation was due to residual [^{14}C]ATP.

Similar results were obtained by passing redissolved trichloroacetic acid precipitates of reaction mixtures through a 1.5 × 25 cm column of Sephadex G-200 equilibrated with 0.02 M Tris-HCl buffer, pH 7.5. The RNA primer and radioactivity eluted at the same position just after the calculated void volume of the column. Short oligoadenylates of the chain length observed in Table VI would elute much later from this column, again indicative of the covalent linkage to the primer RNA.

Evidence that the [^{14}C]oligoadenylate was attached to the 3′-hydroxyl end of the primer polyribonucleotide was ob-

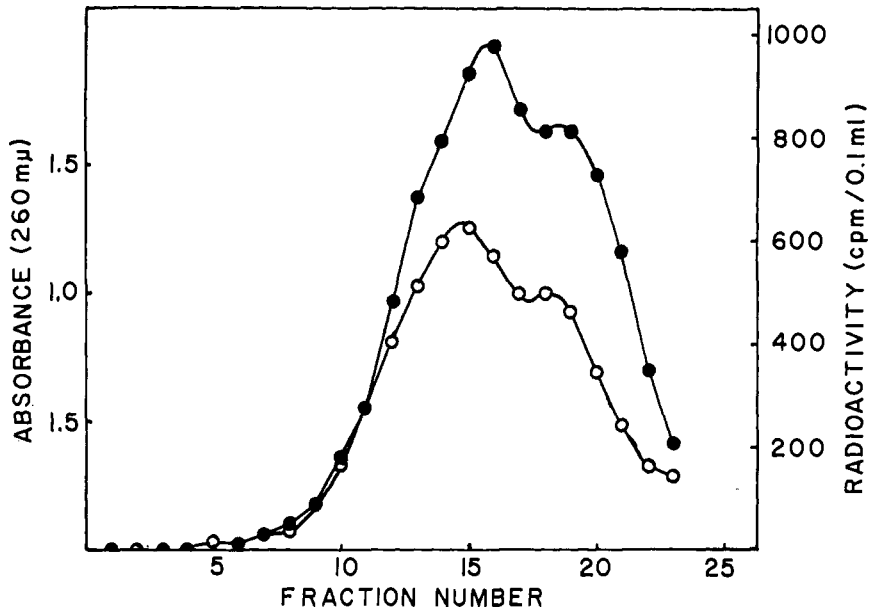

FIGURE 4: Sucrose gradient centrifugation of the RNA primer-associated product. An incubation mixture (0.25 ml) containing the standard components was incubated at 30° for 30 min followed by the addition of 3 ml of cold 5% trichloroacetic acid. The precipitate was washed 3 times with 3 ml of 5% trichloroacetic acid and once with 3 ml of ether. The dried material was dissolved in 0.25 ml of 0.02 M Tris-HCl buffer, pH 7.5, and layered on a 5–25% linear sucrose gradient (in 0.02 M Tris-HCl buffer, pH 7.5) in Spinco SW-39 rotor tube. Centrifugation was carried out for 7 hr at 39,000 rpm. The tubes were punctured at the bottom and 23 fractions were collected. Aliquots (0.1 ml) were used for determining the absorption at 260 mμ (○—○) and for counting radioactivity (●—●). The direction of sedimentation is from right to left. The two peaks of RNA (absorbance at 260 mμ) correspond to the 17S and 26S ribosomal RNA (Breuning and Bock, 1967) as shown by an almost identical absorbance profile obtained by centrifuging ribosomal RNA alone or a zero-time control incubation mixture.

tained by prior digestion of the primer with either pancreatic ribonuclease (forming 3′-phosphate end groups) or yeast ribonuclease (forming 3′-hydroxyl end groups). As shown in Table VII, digestion of either yeast ribosomal RNA or poly(U) with pancreatic ribonuclease for periods up to 30 min before use as a primer led to a decrease in priming function of the RNA to 38% and of the poly(U) to 60%. In contrast, prior digestion with yeast ribonuclease for increasing times up to 30 min led to increased priming function of both ribosomal RNA (139%) and poly(U) (210%). As the yeast ribonuclease hydrolyzes nucleic acids to form oligonucleotides bearing 3′-hydroxyl end groups, the results are consistent with the expected participation of the 3′-hydroxyl group of the primer in the polymerization reaction.

Redissolved trichloroacetic acid precipitates of reaction mixtures were also digested with pancreatic ribonuclease for 16 hr and the resulting oligonucleotides chromatographed on DEAE-cellulose urea columns (Tomlinson and Tener, 1963). This procedure separates oligonucleotides mainly on the basis of the number of phosphate groups present and, therefore, according to degree of polymerization. Chromatography was carried out on 1 × 58 cm columns of DEAE-cellulose equilibrated with 0.02 M Tris-HCl buffer, pH 7.5. The column was eluted with a linear gradient of 0–0.3 M NaCl in 0.02 M Tris-HCl buffer, pH 7.6 containing 7 M urea. Three major radioactivity peaks and several minor components were eluted. As appropriate oligonucleotides of defined chain lengths were not available for calibration of the column, assignment of chain lengths of the eluted radioactive oligonucleotides was not possible. However, comparison of elution positions and NaCl concentration at the point of elution with literature values (Tomlinson and Tener, 1963; Bartos *et al.*, 1963) indicated that these components were of chain length 10 or greater. As these oligonucleotides resulted from pancreatic ribonuclease digestion, they may still contain purine nucleotides and, thus, an exact chain-engtth determination by this method is still not indicative of the oligoadenylate chain length. No radioactive oligonucleotides were eluted from the column without ribonuclease digestion, and, therefore, in addition to giving an approximate chain length, these experiments also lend further support to the covalent linkage of the oligoadenylate to the primer RNA.

Discussion

The yeast poly(A) polymerase resembles in many repects the enzymes studied from other sources. Similar properties include: a requirement for divalent metal ions (Mn^{2+} or Mg^{2+}) and a primer RNA; a specificity for ATP as substrate; and attachment of the poly(A) product to the 3′-hydroxyl end of the primer RNA. The failure of native or denatured DNA to serve as a primer for the yeast enzyme and the inhibition of AMP by the other 3 ribonucleoside triphosphates clearly distinguishes this enzyme from the yeast DNA-dependent RNA polymerase (Frederick *et al.*, 1969) which also catalyzes a DNA-dependent synthesis of polyriboadenylate from ATP.

The yeast and bacterial enzymes are both found associated with ribosomes, although this association appears not to be physiologically significant. The binding of the *E. coli* enzyme to ribosomes has been investigated in some detail by Hardy and Kurland (1966), these authors concluding that the enzyme is merely an adsorbed contaminant resulting from the isolation procedure. The relatively high efficiency of yeast ribosomal RNA, as compared to transfer RNA, synthetic polyribonucleotides or DNA, to function as a primer for the yeast enzyme might imply a functional role, *i.e.*, an involvement in ribosomal RNA metabolism or function. As at least some of the yeast poly(A) polymerase with general requirements and properties similar to the ribosome-associated enzyme can be demonstrated in the ribosome-free cytoplasm at all stages of

growth, a specific attachment to the ribosomes does not seem probable. The fact that the amount of ribosome-attached enzyme decreases with time of growth (toward late logarithmic and stationary phases) suggests an alteration in some component effecting this binding, whether it be the ribosome, poly(A) polymerase, or some other factor.

Recent observations that the DNA-dependent RNA polymerase is a polymeric enzyme in which different subunits play distinct roles in the overall reaction (Burgess *et al.*, 1969; Krakow *et al.*, 1969; Hurwitz and Ishihama, 1969) raises the possibility that the poly(A) polymerase may be a subunit or altered form of the RNA polymerase. Such an altered form might be able to only catalyze elongation of RNA chains by adding AMP residues to the 3′-hydroxyl end, as observed for the poly(A) polymerases, but unable to catalyze initiation of new RNA chains. Keir (1965) has discussed a possible similar origin of the terminal DNA polymerase from the replicative DNA polymerase.

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