Immunoprecipitation of Specific Polysomes Using *Staphylococcus aureus:* Purification of the Immunoglobulin κ Chain Messenger RNA from the Mouse Myeloma MPC11†

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ABSTRACT: A modified procedure for immunoprecipitation of specific polysomes (Mueller-Lantzsch, N., & Fan, H. (1976) *Cell 9,* 579) has been examined as a method for purification of specific mRNAs. Antibody–polysome complexes were recovered by adsorption to inactivated *Staphylococcus aureus* which has a cell wall component (protein A) with a high affinity for immunoglobulins. The technique was evaluated by precipitation of polysomes synthesizing immunoglobulin κ chains from mouse myelomas. Under the immunoprecipitation conditions used neither affinity purified antibody nor formaldehyde-fixed staphylococci degraded polysomes. Using purified sheep anti-mouse immunoglobulin, 10–15% of the polysomes from the myeloma MOPC41A and about 5% from MPC11 (66.2) could be precipitated, values compatible with the amount of κ chain mRNA present in these tumors. Experiments to assess specificity, in which antibody or bacteria were omitted, or in which non-immune sheep immunoglobulin or liver polysomes were substituted, indicated that nonspecific precipitation was about 5–10% of specific precipitation. Kinetic hybridization analysis showed that κ chain sequences were specifically and efficiently enriched during immunoprecipitation. The level of nonspecific precipitation was higher when the scale of the reaction was increased to allow purification of mRNA. By a combination of immunoprecipitation of specific polysomes and size fractionation of polyadenylated RNA, it was possible to isolate intact κ mRNA from MPC11 (66.2); hybridization analysis indicated that this mRNA was about 90% pure.

Many studies of gene function and organization require purified messenger RNAs (mRNAs), for example, as hybridization probes to study the number of corresponding genes and their arrangement. Various size fractionation procedures, in combination with chromatography on oligo(dT)-cellulose or poly(U)[1]-Sepharose, have been used to purify mRNAs with unusual physical properties, such as collagen (Wang et al., 1975) and silk fibroin mRNAs (Suzuki & Brown, 1972), or ones which represent a substantial fraction of the total cellular mRNA, for example, the mRNAs for globin (Aviv & Leder, 1972), ovalbumin (Rosen et al., 1975), casein (Rosen, 1976) and certain immunoglobulin chains (Swan et al., 1972; Mach et al., 1973; Cory et al., 1976; Cowan et al., 1976, Honjo et al., 1976; Tonegawa, 1976). Most mRNAs, however, are neither sufficiently unusual in structure, nor present in high enough concentrations for physical fractionation to yield pure material.

A technique potentially of more general applicability is immunoprecipitation of specific polysomes, an approach which exploits the ability of antibodies raised against a native protein to bind to the nascent polypeptide chains on polysomes (Palacios et al., 1972). The resulting soluble antibody–polysome complexes have usually been isolated by indirect immunoprecipitation, using an anti-immunoglobulin antibody. This "double antibody" technique has been used to substantially purify the mRNAs encoding chicken ovalbumin (Shapiro & Schimke, 1975), chicken vitellogenin (Jost & Pehling, 1976), sheep caseins (Houdebine & Gaye, 1976), rat liver albumin (Taylor & Tse, 1976), mouse immunoglobulins (Schechter, 1974; Legler & Cohen, 1976), and the chicken reticulocyte histone, H5 (Scott & Wells, 1975, 1976). Nonetheless, the efficiency and specificity of the double antibody technique are variable, a likely cause of decreased specificity being entrapment of irrelevant polysomes within immune complexes (Shapiro et al., 1974; Taylor & Tse, 1976).

The specificity of immunoprecipitation might be significantly improved if the antibody–polysome complexes were recovered on a solid-phase adsorbent, which should allow more efficient washing and result in less nonspecific trapping. A scheme in which the second antibody is linked to cellulose has been used to isolate ovalbumin polysomes (Schutz et al., 1977). An alternative solid-phase adsorbent for immune complexes is inactivated *Staphylococcus aureus,* which has a cell wall component, protein A, with high affinity for immunoglobulins of certain classes (Kronvall et al., 1970). With simple protein antigens the *Staphylococcus* adsorption method has proven to be much more rapid and to give far less background precipitation than the double antibody approach (Kessler, 1975; Goding, 1978). The potential of this approach with polysomes has been demonstrated by the immunoprecipitation of polysomes synthesizing a Moloney leukemia viral protein, where a double antibody procedure had proven inadequate (Mueller-Lantzsch & Fan, 1976).

We have investigated the *S. aureus* approach in detail, using as a model system microsomal polysomes from the murine plasmacytoma MOPC41A, which secretes a κ chain. It is possible to isolate mRNA coding for this κ chain by size fractionation (Mach et al., 1973; Cory et al., 1976) and to prepare substantially pure cDNA[1] on this template (Bernard et al., 1977). We have used this probe to follow the purification of κ mRNA sequences during immunoprecipitation. We found that the immunoprecipitation technique was specific and efficient on an analytical scale (such as was used by Mueller-Lantzsch & Fan (1976)), but exhibited reduced specificity in

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[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; cDNA, complementary DNA; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; Ig, immunoglobulin; poly(A), poly(adenylic acid); poly(U), poly(uridylic acid).

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the larger scale reactions required for purification of mRNA. Nonetheless, it was possible to use this procedure in conjunction with size fractionation to purify the κ light chain mRNA from another myeloma, MPC11 (66.2), in which this mRNA represents less than 5% of the total mRNA (Stavnezer et al., 1974; Kuehl et al., 1975).

Methods

Antigens and Antisera. Mouse immunoglobulins were isolated from ascitic fluid of female BALB/c mice bearing intraperitoneal tumors of the myelomas MPC11 (Laskov & Scharff, 1970) and HPC76 (obtained from Dr. N. Warner). MPC11 immunoglobulin ($IgG_{2b}(\kappa)$) was purified by two cycles of affinity chromatography on protein A–Sepharose (Pharmacia). After the clarified ascitic fluid had been loaded, the column was thoroughly washed with 0.15 M NaCl and 0.2 M NaSCN (to remove loosely bound material); the bound immunoglobulin was then eluted with 0.1 M acetic acid in 0.15 M NaCl, dialyzed against PBS (0.15 M NaCl, 0.02 M sodium phosphate, pH 7.3), and stored frozen. The protein was homogeneous as assessed by electrophoresis on $NaDodSO_4$–polyacrylamide gels. The HPC76 protein ($IgM(\kappa)$) was purified by two low salt precipitations (by dialysis against 0.01 M NaCl) followed by chromatography on protein A–Sepharose, to which this IgM myeloma protein binds (MacKenzie et al., 1978). A linear gradient of 0–3 M NaSCN was applied, and the HPC76 protein eluted at approximately 0.5 M. An immunoglobulin fraction of normal sheep serum was obtained by two precipitations with 33% $(NH_4)_2SO_4$ (Stelos, 1967).

Sheep anti-mouse IgG was raised by three subcutaneous injections of 1 mg of purified MPC11 immunoglobulin in Freund's complete adjuvant (Difco) at intervals of 1 month. One week prior to bleeding, a booster injection of 1 mg of MPC11 immunoglobulin in PBS was administered. Rabbit anti-sheep Ig was the generous gift of Dr. J. W. Goding.

Purification of Antibodies by Affinity Chromatography. The MPC11 and HPC76 proteins were coupled to CNBr-activated Sepharose (Pharmacia) at a concentration of 5–10 mg of protein per mL of packed Sepharose. After 95% coupling had been achieved, any remaining unreacted groups were blocked by reaction with ethanolamine chloride, pH 8.0. The conjugated Sepharose was washed with 3.5 M NaSCN and PBS. During affinity chromatography, antiserum was left in contact with the antigen–Sepharose for at least an hour. The column was then washed with 0.15 M NaCl and 0.2 M NaSCN (both 1 mL/mL of loaded serum) and again with 0.15 M NaCl (4–5 times the loaded volume). Finally, antibody was eluted with either 3.5 M NaSCN, or with 0.08 M citric acid/0.04 M Na_2HPO_4, pH 3.0 (see Results).

Purification of sheep anti-mouse IgG antibodies on MPC11–Sepharose routinely gave about 1 mg of specific antibody per mL of antiserum. Anti-κ chain activity was recovered from the anti-IgG preparation by passage over an HPC76-Sepharose column (since the HPC76 protein has a μ heavy chain, only the anti-κ activity is adsorbed). Rabbit anti-sheep Ig antibody was purified on sheep Ig–Sepharose.

Polysome Preparation. The myelomas MOPC41A (Potter et al., 1964) and MPC11 (66.2) (Kuehl & Scharff, 1974) were maintained in female BALB/c mice by subcutaneous injection of small pieces of tissue. Polysomes were isolated from microsomes (the major site of immunoglobulin synthesis) as previously described (Cory et al., 1976), with the modification that all buffers used contained 0.3 M KCl and 3 mM glutathione (see Results). After detergent release, the polysomes were purified by sedimentation for 4–5 h at 50 000 rev/min (Beckman Ti60 rotor) through a cushion of 9 mL of 2 M sucrose in 0.3 M KCl, 5 mM $MgCl_2$, 0.05 M Tris-Cl, pH 7.54, 3 mM glutathione. The polysomes were resuspended in Na300 (0.3 M NaCl, 5 mM $MgCl_2$, 0.05 M Tris-Cl, pH 7.54) at 100–200 A_{260}/mL, and stored at −80 °C; they were stable for several months. Liver microsomal polysomes were prepared similarly, from mice fasted for 16 h.

^3H-labeled polysomes were prepared from mice injected intraperitoneally with 0.5 mCi of [5-^3H]uridine at 16 and 40 h prior to sacrifice. Typically, tumor polysomes had a specific activity of 25 000 cpm/A_{260} unit and liver polysomes 90 000 cpm/A_{260} unit.

Preparation of the Bacterial Adsorbent. Staphylococcus aureus strain Cowan 1 (NCTC 8530), obtained from Dr. J. B. Egan, was grown in 5CY broth (Novick, 1963). The bacteria were washed, heat killed, and fixed in formaldehyde as described by Kessler (1975) and stored at −20 °C (Cullen & Schwartz, 1976). To remove any soluble protein A or ribonuclease, the bacteria were thawed just before use, washed three times in 10 volumes of NET buffer (0.15 M NaCl, 5 mM EDTA, 0.05 M Tris-Cl, pH 7.4, 0.05% NP40, 0.02% sodium azide), three times in Na300 buffer containing 0.5% NP40 and 0.5% sodium deoxycholate, and finally resuspended in this buffer.

Immunoprecipitation of Polysomes. Polysomes and antibodies were deaggregated just prior to use by centrifugation for 10 min at 12 000 rev/min (Sorvall HB4 rotor). In analytical experiments (involving less than 10 A_{260} units of polysomes), incubation mixtures contained 5–10 A_{260} units of ^3H-labeled polysomes per mL in Na300 buffer plus 0.5% NP40, 0.5% sodium deoxycholate, and varying amounts of antibody. After 1 h at 0 °C the staphylococci (in Na300 + 0.5% NP40 + 0.5% sodium deoxycholate) were added and incubation continued for 10 min. The incubation mixtures were then layered over discontinuous sucrose gradients consisting of 1.5 mL of 0.5 M sucrose over 2.5 mL of 1 M sucrose, both in Na300 buffer containing 1% NP40 and 1% sodium deoxycholate. The bacteria were sedimented by centrifugation for 2 h at 4800g. Polysomal RNA was released from the bacterial pellet by resuspending it in 500 μL of 1.5% $NaDodSO_4$, 0.15 M NaCl, 20 mM EDTA, 10 mM Tris-Cl, pH 7.5. (If polysome precipitation was to be measured optically, the same buffer without $NaDodSO_4$ was used.) The bacteria, which do not lyse, were then removed immediately, by centrifugation at 3000g for 10 min, and the radioactivity in the supernatant was determined.

In large scale precipitations for preparation of mRNA (greater than 200 A_{260} units of polysomes), the following modifications were made. EGTA (2 mM) and 0.2 mM thymidine 3′,5′-diphosphate (Calbiochem), potent inhibitors of micrococcal nuclease (Cuatrecasas et al., 1967), were added to all buffers. The bacteria–polysome complexes were sedimented through cushions of 4.5 mL of 0.5 M sucrose over 7.5 mL of 1 M sucrose for 17 min at 12 000 rev/min in a Sorvall HB4 rotor. The pellet was resuspended in the incubation buffer and the complex resedimented. Polysomal RNA was released from the pellet using 8 mL of $NaDodSO_4$ buffer/mL of staphylococci and the bacteria were removed as above. The supernatant was then treated with proteinase K (0.6 mg/mL) and extracted with phenol as previously described (Cory et al., 1976). The RNA was recovered by ethanol precipitation and reprecipitated twice more. Poly(A)-containing RNA was isolated from polysomal RNA by 2 cycles of adsorption to oligo(dT)-cellulose with an intermediate heat step as described previously (Cory & Adams, 1975); 1.5–2.5% of the RNA bound.

Hybridization Analysis of Immunoprecipitated RNA. The

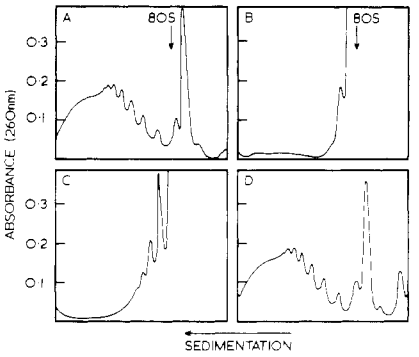

FIGURE 1: Removal of ribonuclease from antibodies. Five A_{260} units of MOPC41A polysomes was incubated with various antibody preparations (0 °C, 1 h) and then layered over 15–40% (w/w) linear sucrose gradients in Na300 buffer and centrifuged at 40 000 rev/min (Beckman SW40 Rotor) for 85 min at 4 °C. Incubation mixes contained: (A) no antibody; (B) 100 μL of antiserum; (C) 100 μg of antibody, affinity purified without a 0.2 M NaSCN wash; (D) 100 μg of antibody, purified with a 0.2 M NaSCN wash.

κ chain mRNA from MOPC41A was isolated by size fractionation (Cory et al., 1976) and ^{3}H-labeled cDNA synthesized using reverse transcriptase (kindly provided by Dr. J. W. Beard) and a primer complementary to the 3′ terminus of this mRNA, pdT_8C, as described previously (Bernard et al., 1977). RNA-excess hybridization was carried out in sealed, siliconized glass capillaries. Reaction mixtures (50 μL) contained 0.6 M NaCl, 0.01 M Tris-Cl, pH 7.1, 2 mM EDTA, approximately 500 cpm of [^{3}H]cDNA, and up to 800 μg/mL RNA. Control samples without RNA were included. Samples were boiled for 1 min, incubated at 66 °C for 10–85 h and frozen. Each was then treated with the single-strand specific nuclease, S1, in a 0.5-mL reaction mixture containing 30 mM sodium acetate, pH 4.6, 50 mM NaCl, 1 mM $ZnSO_4$, 5% glycerol, 10 μg of denatured calf thymus DNA, and 10 units of S1 nuclease (Sigma). After digestion at 45 °C for 30 min, acid-insoluble radioactivity was determined.

Results

Polysome Integrity. Intact mRNA can be isolated only from undegraded polysomes. Following the observations of Ramsey & Steele (1976) and Eker & Pihl (1971) on rat liver polysomes, we found that large polysomes could reproducibly be obtained from mouse myelomas and liver, if all buffers used during isolation contained 0.3 M KCl and 3 mM glutathione. For example, Figure 1A shows the profile of polysomes isolated from the myeloma MOPC41A. Using this procedure polysomes with a mean size of greater than 6 ribosomes could also be isolated from the myelomas MPC11, MPC11 (66.2) MOPC315, and BFPC61.

Integrity of polysomes provided a sensitive test for ribonuclease activity in antibody and staphylococci preparations. Figure 1B shows that polysomes incubated with an untreated sheep antiserum were almost completely degraded. We found, however, that purification of the antibody on an antigen–Sepharose column removed all detectable ribonuclease activity, if the affinity column had been thoroughly washed with 0.2 M NaSCN prior to elution (Figure 1D). Antibody eluted without this step contained substantial ribonuclease activity (Figure 1C). This stringent washing step, which presumably removes loosely bound material, nonspecifically adsorbed to the column, allowed routine purification of antibody free of ribonuclease.

As *Staphylococcus aureus* secretes the potent micrococcal nuclease (Cuatrecasas et al., 1967), it was critical to establish

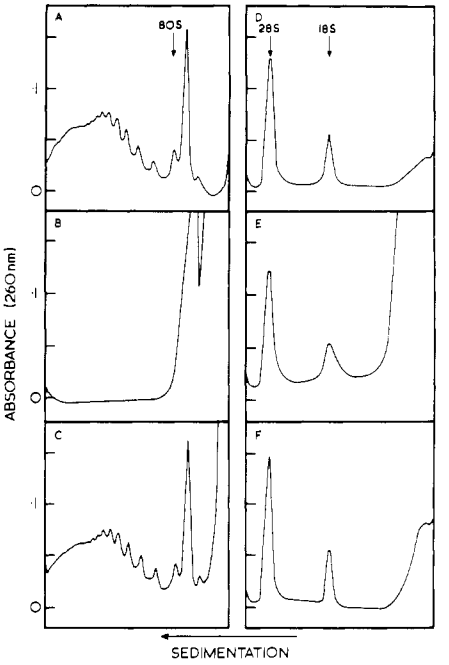

FIGURE 2: Test for ribonuclease in preparations of staphylococci. Five A_{260} units of MOPC41A polysomes was incubated (1 h, 0 °C) with 100 μL of packed staphylococci, in a reaction mixture containing 500 μL of Na300. Incubation mixtures contained: (A and D) no added staphylococci; (B and E) untreated staphylococci; (C and F) formaldehyde-fixed staphylococci washed with NET and Na300. To examine the polysome integrity (A–C), the staphylococci were removed by centrifugation, and the supernatants fractionated as described in Figure 1. To examine the integrity of the rRNA (D–F), EDTA and $NaDodSO_4$ were added to final concentrations of 50 mM and 0.5%, respectively, the staphylococci were removed, and 100 μL of the supernatants was layered over 10–30% (w/w) linear glycerol gradients containing 0.01 M NaCl, 0.01 M triethanolamine chloride, pH 7.5, 1 mM EDTA and centrifuged at 40 000 rev/min (Beckman SW40 rotor) for 11 h at 15 °C.

that the fixed bacteria contained no residual nuclease activity. As expected, the untreated bacteria completely degraded polysomes, as shown in Figure 2B. However, bacteria fixed with formaldehyde and washed with EDTA to chelate calcium ions, which are required by micrococcal nuclease for activity (Cuatrecasas et al., 1967), exhibited no detectable ribonuclease activity (Figure 2C). Several batches of treated staphylococci were shown to be free of ribonuclease activity. Since the $NaDodSO_4$ treatment used to dissociate polysomal RNA from the bacterial surface might conceivably liberate ribonuclease from within the staphylococci or the polysomes, polysomes were disrupted in the presence of staphylococci, and the integrity of the released myeloma ribosomal RNA was examined (Figures 2D–F). Untreated staphylococci caused marginal degradation of the rRNA, which is presumably protected prior to $NaDodSO_4$ disruption (Figure 2E). The formaldehyde fixed and washed bacteria, however, caused no degradation (Figure 2F), demonstrating that no further active ribonuclease was released. Some 2–4S material was released from the bacteria (compare Figures 2D and 2F), but control experiments, without myeloma polysomes, indicated that no high molecular weight RNA was released.

The Efficiency and Specificity of Immunoprecipitation. Preliminary experiments indicated that maximum precipitation of protein antigens was achieved using 0.9 μL of packed staphylococci per μg of antibody. This ratio, which was confirmed for precipitation of polysomes (Figure 4), was used in all subsequent studies.

To define the optimal conditions for immunoprecipitation,

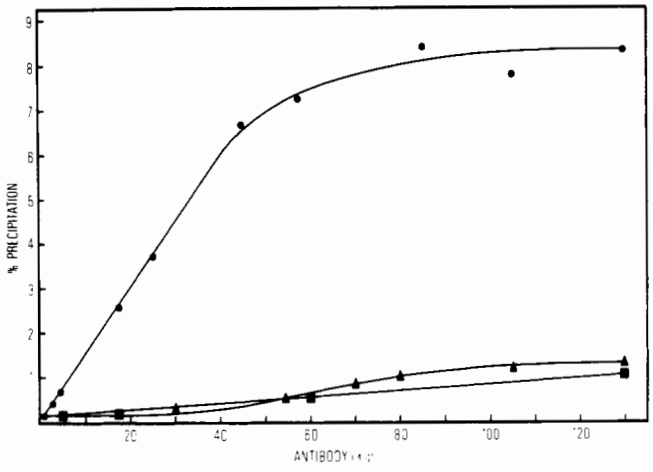

FIGURE 3: Effect of antibody concentrations on the immunoprecipitation of ^{3}H-labeled polysomes. MOPC41A polysomes were incubated with the indicated amounts of sheep anti-mouse IgG antibody (●—●) or non-immune sheep Ig (■—■), and liver polysomes with sheep anti-mouse IgG (▲—▲).

TABLE I: Requirements for Immunoprecipitation of MOPC 41A Polysomes.

incubation	precipitation (%)
complete reaction[a]	8.7
− staphylococci[b]	0.1
− anti-Ig[b]	0.4
− anti-Ig and staphylococci[b]	0.1
+ non-immune sheep Ig[c]	0.6
+ protein A[d]	0.7

[a] ^{3}H-labeled MOPC41A polysomes were immunoprecipitated as described, using 100 μg of sheep anti-mouse IgG antibody and 90 μL of packed staphylococci per A_{260} unit of polysomes. [b] The indicated components have been omitted from the complete reaction. [c] Non-immune sheep Ig, 100 μg, was used in place of antibody. [d] Protein A, (Pharmacia) 100 μg, was added to the complete reaction 5 min prior to the staphylococci.

analytical scale experiments were performed using polysomes labeled with [^{3}H]uridine. Figure 3 shows that with increasing amounts of sheep anti-mouse IgG, MOPC41A polysomes were precipitated to a plateau level of 8.5%. With different batches of polysomes, this value ranged up to 11.7%; these amounts are compatible with the fraction of microsomal mRNA represented by this κ chain mRNA (Stavnezer et al., 1974; and our unpublished experiments). A similar level of precipitation was obtained using purified anti-κ chain antibody.

Evidence for specificity is also provided in Figure 3, which shows that sheep-anti mouse IgG precipitated no more than 1% of liver polysomes (15% of the liver polysomes were precipitable with antialbumin). Moreover, less than 1% of MOPC41A polysomes were precipitated when non-immune sheep Ig (or antialbumin) was substituted for specific antibody.

Omitting antibody or bacteria from the system eliminated most precipitation (Table I), indicating that both components are required. Moreover, the addition of soluble protein A prior to the staphylococci strongly inhibited precipitation, demonstrating that antibody–polysome complexes are specifically adsorbed to the bacterial surface, rather than being nonspecifically entrapped.

Since physical entrapment of irrelevant polysomes within polysome–antibody complexes has been implicated as a major

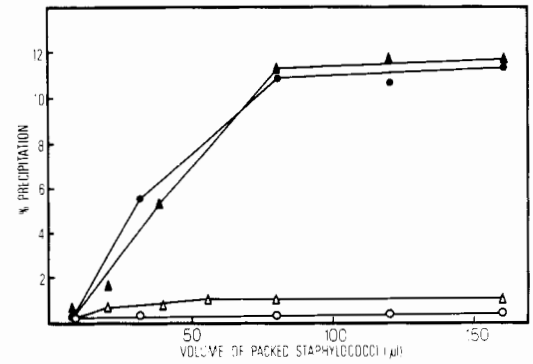

FIGURE 4: Immunoprecipitation of polysomes from a mixture of myeloma and liver polysomes. Equal amounts of ^{3}H-labeled liver and unlabeled MOPC41A polysomes were mixed and immunoprecipitated with 100 μg of sheep anti-mouse IgG per A_{260} unit of MOPC41A polysomes, and the indicated amounts of staphylococci. Precipitation of MOPC41A polysomes (▲—▲ and ●—●) was assessed optically and that of the liver polysomes (△—△ and ○—○) by radioactivity. The absorbances of samples containing the same amounts of antibody plus staphylococci, but without polysomes, were subtracted from the experimental points. Although this background absorbance ranged to as high as four times that of the immunoprecipitated polysomes, this simple measurement was found to be satisfactory since the calculated precipitation was comparable to that observed using ^{3}H-labeled myeloma polysomes. Antibodies were eluted during affinity purification using either 3.5 M NaSCN (▲—▲ and △—△) or the citric acid/sodium phosphate buffer (●—● and ○—○) as described in Methods.

cause of nonspecific precipitation (Shapiro et al., 1974; Taylor & Tse, 1976), a critical test of specificity is to measure the amount of irrelevant polysomes coprecipitated with relevant polysomes. Figure 4 shows that the amount of ^{3}H-labeled liver polysomes precipitated during immunoprecipitation of MOPC41A polysomes from a mixture of the two is comparable with the level observed for liver polysomes alone (Figure 3). This demonstrates that entrapment of polysomes in bacteria–antibody–polysome complexes is negligible.

Figure 4 also shows that a lower level of nonspecific precipitation resulted when the antibodies used had been recovered from the affinity column with citric acid/sodium phosphate buffer as an eluting agent. This probably reflects the reduced level of aggregation of eluted antibody caused by this eluting agent compared with 3.5 M NaSCN.

Comparison with the Double Antibody Procedure. The efficiency and specificity of precipitation of immunoglobulin synthesizing polysomes by the double antibody technique were examined. A polysome mixing experiment was again performed, in which unlabeled MOPC41A and ^{3}H-labeled liver polysomes were incubated with a constant amount of sheep anti-κ antibody (determined as above) and increasing amounts of rabbit anti-sheep Ig antibody (Figure 5). Whilst the precipitation of MOPC41A polysomes was as efficient as that observed using staphylococci, the level of nonspecific precipitation was at least three times higher (compare Figures 4 and 5).

Is Specific Messenger RNA Enriched by Immunoprecipitation? Having determined the optimal conditions for immunoprecipitation, we used a kinetic hybridization method (Bishop et al., 1974) to confirm that specific mRNA was enriched. Polysomal RNA was extracted from immunoprecipitates and also from the polysomes which remained unprecipitated. Figure 6 shows the kinetics of hybridization of these RNA samples to ^{3}H-labeled MOPC41A κ cDNA. The $ER_0t_{1/2}$ value of total polysomal RNA was 12.5-fold higher than that of immunoprecipitated RNA, indicating that immunoprecipitation had enriched the κ mRNA 12.5 fold.

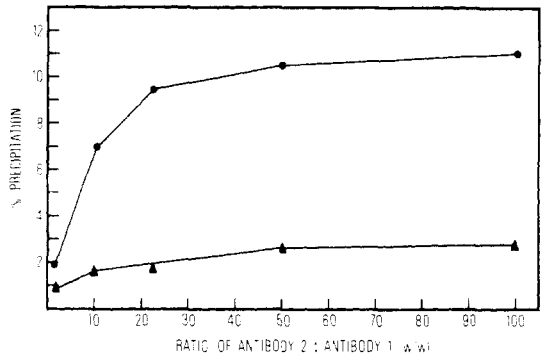

FIGURE 5: Immunoprecipitation of polysomes using the double antibody technique. Equal amounts of unlabeled MOPC41A and ^{3}H-labeled liver polysomes were mixed and incubated (1 h, 0 °C) with 25 μg of sheep anti-κ (antibody 1) per A_{260} unit of MOPC41A polysomes. The indicated amounts of purified rabbit anti-sheep Ig (antibody 2) were added and incubation continued for an additional hour. Immune complexes were sedimented by centrifugation (3 h, 4800g) through cushions of 1.5 mL of 0.5 M sucrose over 2.5 mL of 1 M sucrose in Na300 + 1% NP40 + 1% sodium deoxycholate. Pellets were resuspended in 0.1 M NaOH and precipitation assessed optically (Schechter, 1974) and then counted for radioactivity. MOPC41A polysomes (●—●); ^{3}H-labeled liver polysomes (▲—▲).

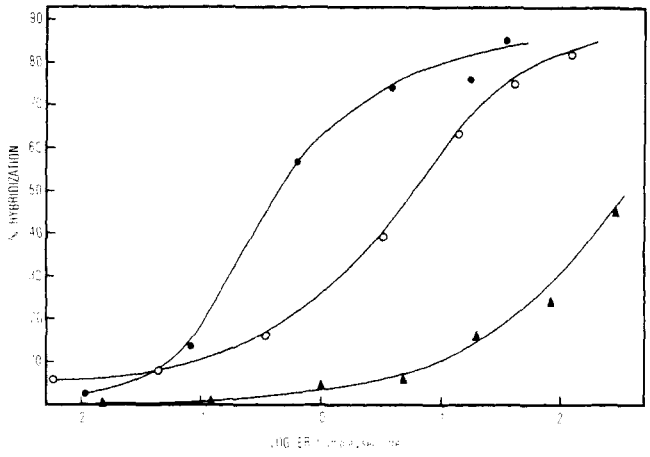

FIGURE 6: Kinetic hybridization analysis of immunoprecipitated MOPC41A RNA. MOPC41A polysomal RNA enriched (●—●) and depleted (▲—▲) by immunoprecipitation and total polysomal RNA (○—○) were hybridized with ^{3}H-labeled MOPC41A κ cDNA. The data are expressed as the percentage of cDNA resistant to S1 nuclease treatment (% hybridization) vs. the product of the RNA concentration × time, normalized to 0.12 M phosphate buffer (ER_0t) (Britten et al., 1974). The background S1 nuclease resistance of the cDNA, determined in samples without added RNA, varied between 5 and 10%, and has been subtracted from all experimental points.

Moreover, the κ mRNA sequences were very efficiently precipitated: the $ER_0t_{1/2}$ value of the depleted polysomal RNA sample (about 3×10^{-2}) indicates that less than 5% remained unprecipitated.

Purification of the κ Chain mRNA from MPC11 (66.2). Having established the efficacy of the technique, we then employed it in an attempt to purify the κ chain mRNA from another light chain secreting myeloma, MPC11 (66.2). The optimal amount of anti-κ antibody per A_{260} unit of MPC11 (66.2) polysomes was determined as in Figure 3. In these analytical scale experiments about 5% of the polysomes were precipitated, a level compatible with the amount of κ chain mRNA present in this tumor (Stavnezer et al., 1974; Kuehl et al., 1975; Storb et al., 1977). However, when the scale of the immunoprecipitation was increased 200-fold to permit isolation of the κ chain mRNA, approximately 12% of the polysomes

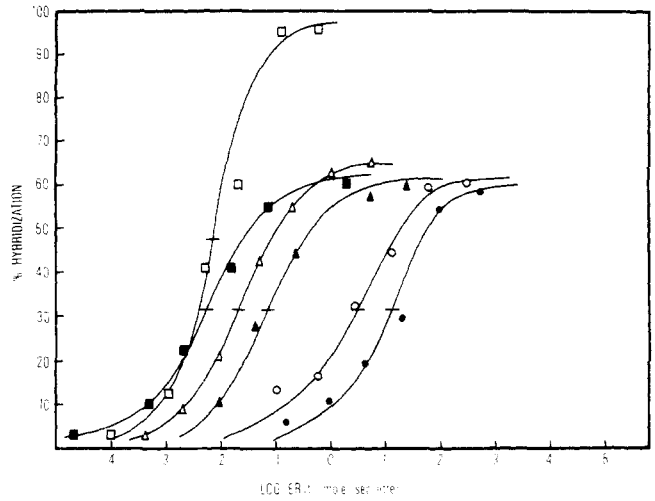

FIGURE 7: Kinetic hybridization analysis of MPC11 (66.2) RNA. RNA samples at various stages of purification were hybridized with MOPC41A κ cDNA. Data are presented as described in Figure 6. Total polysomal RNA (●—●), immunoprecipitated polysomal RNA (○—○), total polyadenylated RNA (▲—▲), immunoprecipitated polyadenylated RNA (△—△), immunoprecipitated polyadenylated RNA, fractionated by gradient centrifugation (■—■), and size fractionated MOPC41A κ mRNA (□—□).

were precipitated, suggesting that specificity had been reduced.

To assess enrichment, MPC11 (66.2) RNA samples from immunoprecipitates were hybridized with MOPC41A κ cDNA (Figure 7); as noted by Kuehl et al. (1975), only 60% of this cDNA hybridizes with MPC11 (66.2) mRNA, reflecting the amino acid sequence differences in the variable regions of the two light chains (Gray et al., 1967; Smith, 1973). A comparison of the $ER_0t_{1/2}$ values (Figure 7 and experiment 4, Table II) observed for immunoprecipitated and total polysomal RNA samples (3.1 and 12, respectively) indicates that immunoprecipitation had enriched the κ mRNA 4-fold. Similarly, the polyadenylated immunoprecipitated RNA was enriched approximately 3.5-fold compared with total mRNA.

The immunoprecipitated mRNA was fractionated by sedimentation on a glycerol gradient. A comparison with total mRNA (Figure 8) shows that there was a prominent 13S peak of RNA that had been substantially enriched by immunoprecipitation. This RNA cosedimented with a purified κ mRNA marker suggesting that despite the rigorous immunoprecipitation conditions the RNA was substantially intact. This was confirmed by electrophoresis of RNA from the peak on polyacrylamide gels in 98% formamide (not shown). RNA obtained from this major peak was shown to hybridize with an $ER_0t_{1/2}$ value of $5\text{–}6 \times 10^{-3}$ (experiment 4, Table II) which is 20% lower than the value for our MOPC41A mRNA standard, indicating that this mRNA preparation was substantially pure.

In two large scale precipitations of MPC11 (66.2) polysomes, the enrichment of κ mRNA observed was 4- and 8-fold (experiments 4 and 5, Table II), compared with a possible 15–20-fold. Reduced specificity was also observed for large scale MOPC41A preparations; experiments 2 and 3 in Table II show that the enrichment obtained was 3- and 8-fold, in contrast with the 12.5-fold observed in an analytical scale experiment (experiment 1, Table II).

Discussion

The goals of this work were to examine the efficacy of inactivated *S. aureus* as a solid-phase adsorbent for the precip-

TABLE II: Enrichment of κ mRNA by Immunoprecipitation and Size Fractionation.[a]

expt no.[b]	myeloma	RNA sample	$ER_0t_{1/2}$[c]	enrichment[d]
1	MOPC41A	total polysomal RNA	5	1
		immunoprecipitated polysomal RNA	4×10^{-1}	12.5
		total polysomal RNA, depleted by immunoprecipitation	3×10^{2}	*e*
2	MOPC41A	total polysomal RNA	5.2	1
		immunoprecipitated polysomal RNA	1.7	3
		total polyadenylated RNA	3.7×10^{-2}	140
		immunoprecipitated polyadenylated RNA	1.2×10^{-2}	433
		total polyadenylated RNA, size fractionated	5.9×10^{-3}	881
		immunoprecipitated polyadenylated RNA, size fractionated	4.5×10^{-3}	1156
3	MOPC41A	total polysomal RNA	4	1
		immunoprecipitated polysomal RNA	5.3×10^{-1}	8
4	MPC11 (66.2)	total polysomal RNA	12	1
		immunoprecipitated polysomal RNA	3.1	4
		total polyadenylated RNA	7.9×10^{-2}	152
		immunoprecipitated polyadenylated RNA	2.4×10^{-2}	500
		immunoprecipitated polyadenylated RNA, size fractionated	5.3×10^{-3}	2264
5	MPC11 (66.2)	immunoprecipitated polyadenylated RNA	9.8×10^{-3}	1225
6	MOPC41A	size fractionated polyadenylated RNA[f]	6.5×10^{-3}	

[a] Determined by kinetic hybridization analysis as described in Methods and Figure 6. [b] The data presented for experiments 1 and 4 are taken from Figures 6 and 7, respectively. Experiment 1 is an analytical scale experiment; experiments 2–6 are preparative scale. [c] The value of ER_0t at which half-maximum hybridization is obtained. [d] Determined by dividing the $ER_0t_{1/2}$ value into the $ER_0t_{1/2}$ value for total polysomal RNA in that experiment. Enrichment in experiment 5 is determined relative to experiment 4. [e] Less than 5% of κ mRNA sequences remain unprecipitated. [f] Isolated as described by Cory et al. (1976).

itation of antibody–polysome complexes, in order to facilitate purification of those mRNA species present in low concentrations, and to apply the technique to the purification of the κ chain mRNA from the myeloma MPC11 (66.2).

It was of immediate importance to establish that ribonuclease activity could be efficiently removed from both antibody and staphylococci preparations. Previously ribonuclease has been removed from rabbit antisera by ion-exchange chromatography (Palacios et al., 1972). However, as immunoglobulins display heterogeneous isoelectric points (Porter, 1960), it is not clear whether this approach can be applied to all antisera. We found that chromatography on an antigen affinity column effectively removed ribonuclease if a stringent washing step was included. This approach should be more generally applicable, and allow purification of specific antibodies in a single step.

Nuclease in preparations of staphylococci did not prove to be a problem. Residual ribonuclease present in the formaldehyde-fixed bacteria was inactivated by washing in an EDTA-containing buffer, presumably because micrococcal nuclease requires calcium ions (Cuatrecasas et al., 1967). Moreover, the immunoprecipitation buffers contained magnesium ions, which are inhibitory to this enzyme. That ribonuclease had been effectively removed from all components of the immunoprecipitation system was demonstrated by our isolation of apparently intact 13S κ mRNA (Figure 8).

The specificity of the precipitation procedure was demonstrated by the following results. (a) Precipitation required the presence of both staphylococci and specific antibody (Table I). If either was omitted, or if specific antibody was replaced by non-immune Ig, less than 0.6% precipitation was observed. Furthermore, precipitation of immune complexes results from a specific interaction between antibody and cell wall protein A, as it could be inhibited by soluble protein A. (b) Less than 1% of liver polysomes were precipitated with anti-Ig and staphylococci (Figures 3). (c) When a mixture of myeloma and liver polysomes was treated with anti-Ig, again less than 1% of the liver polysomes were precipitated (Figure 4). (d) Kinetic

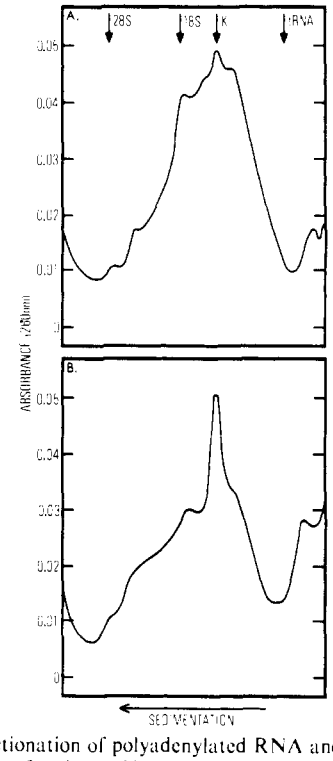

FIGURE 8: Fractionation of polyadenylated RNA and MPC11 (66.2). RNA samples were fractionated by sedimentation at 4 °C for 6 h at 56 000 rev/min in a Beckman SW56 rotor through 10–30% (w/w) glycerol gradients in 0.01 M NaCl, 0.01 M triethanolamine chloride, pH 7.5, 1 mM EDTA. Before loading, the RNA was heated at 65 °C in 100 μL of gradient buffer for 5 min and then quickly chilled. The positions of 28S and 18S rRNA, 4S tRNA and MOPC41A κ mRNA markers were determined from parallel gradients. (A) Total polyadenylated RNA; (B) immunoprecipitated polyadenylated RNA.

hybridization analysis (Figure 6) indicated that immunoprecipitation of MOPC41A polysomes enriched the κ mRNA sequences approximately tenfold, as would be expected if the 8.5–11.7% precipitation observed was largely specific. More-

over, the precipitation procedure was very efficient, leaving less than 5% of the κ mRNA sequences unprecipitated.

In agreement with the results of Mueller-Lantzsch & Fan (1976), the staphylococcal adsorption technique exhibited higher specificity than the double antibody procedure (Figure 5). The increased specificity is probably due to the more efficient washing permitted by adsorption compared with cross-linking (Shapiro et al., 1974; Taylor & Tse, 1976; Schutz et al., 1977).

We used a kinetic hybridization assay to follow the purification of the κ chain mRNA from MPC11 (66.2). We found that, when we increased the scale of the immunoprecipitation to allow purification of the mRNA, the enrichment observed for the κ mRNA varied between 4- and 8-fold (Table II, experiments 4 and 5), in contrast with the 20-fold enrichment expected from the concentration of κ mRNA in this tumor (Stavnezer et al., 1974; Kuehl et al., 1975). When mRNA which had been enriched by immunoprecipitation was fractionated by gradient centrifugation, mRNA was obtained which was 10–20% purer than our MOPC41A κ mRNA standard. We estimate that the standard is about 75% pure, based on sequencing studies (Bernard et al., 1977), cell-free translation, gel electrophoresis, and fingerprint analysis (Cory et al., 1976). Certainly the standard behaved as a single component in kinetic hybridization (Figure 7). Hence we conclude that the MPC11 (66.2) κ mRNA preparation was about 90% pure.

The immunoprecipitation technique, in combination with size fractionation of polyadenylated RNA, should be applicable in many situations where small amounts of highly purified mRNA are required. For example, the mRNA could serve as an effective hybridization probe in gene counting studies, in the analysis of gene arrangement by restriction endonuclease mapping, or in screening bacterial colonies (or phage plaques) to identify clones bearing specified DNA sequences.

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