Use of Antibodies for the Isolation of Biologically Pure Messenger Ribonucleic Acid from Fully Functional Eukaryotic Cells†

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ABSTRACT: An immunological procedure for the preparation of biologically pure mRNA from cells that contain a multitude of different mRNAs is described. The double antibody technique was used employing purified antibodies prepared by means of specific immunoadsorbents. The amount of mRNA obtained from immune-precipitated polysome was within the expected theoretical yield and the procedure could be run on a large scale (25,000 A_{260} units of polysomes can be processed in one batch). This procedure was used for the isolation from a mouse myeloma of the mRNA coding for immunoglobulin light chain (see also Schechter, I. (1973), *Proc. Nat. Acad. Sci. U. S. 70*, 2256). The biological purity of this mRNA (*i.e.*, the capacity to program the synthesis only of L chain in a cell-free system) was found to be ≥95%. Because unique features of the antigen and antibodies were not required in this system, it seems that this procedure would serve as a general solution for the isolation of biologically pure mRNA molecules from fully functional eukaryotic and prokaryotic cells.

One of the major properties that characterizes specialized cells is their capacity to exhibit preferential synthesis of a given protein, be it an enzyme, hormone, structural, or secretory protein. It seems, therefore, that a protein unique for a given cell type could serve as a tool for getting an insight into the molecular basis of cell specialization and of the process of differentiation. With the aid of mRNA, coding for this particular protein, it would be possible to study the control of protein production: (1) at the gene level, by hybridization of the mRNA or its highly labeled DNA transcript (to be prepared from the mRNA by using reverse transcriptase) with DNA obtained from cells of different tissues and at different stages of maturation; (2) at the transcription level, by using the highly labeled DNA transcript to measure mRNA contents in cells and cell fractions; (3) at the translation level, by looking for factors that would affect specifically the rate of mRNA translation in cell-free systems. Meaningful results, however, would be obtained by using biologically pure mRNA, that is, mRNA that in a cell-free system would program the synthesis only of the protein in question.

The antibody approach appears to be, at the present time, the only general solution for the preparation of biologically pure mRNA from cells that contain a large number of different mRNAs. The application of the double antibody technique for the specific isolation of polysomes engaged in the synthesis of β-galactosidase was reported already by Cowie *et al.* (1961). Subsequently, it has repeatedly been shown in several systems that antibodies can bind to nascent chains on polysomes (Warren and Goldthwait, 1962; Warren and Peters, 1965; Duerre, 1967; Williamson and Askonas, 1967; Allen and Terrence, 1968; Hartlief and Koningsberger, 1968; Schubert, 1968; Schubert and Cohn, 1968; Holme *et al.*, 1971a,b; Takagi and Ogata, 1971; Clayton *et al.*, 1972; Hamlin and Zabin, 1972; Palacios *et al.*, 1972; Sarkar and Moscona, 1973). Nevertheless, previous attempts to purify mRNA from immune-precipitated polysomes have met with limited success (Uenoyama and Ono, 1972; Delovitch *et al.*, 1972). Recently Palacios *et al.* (1973) reported on the use of immunoadsorbents for preparing an RNA fraction that was highly enriched for ovalbumin mRNA. In the latter study, however, biological purity of the mRNA has not yet been sufficiently established since the procedure employed to isolate the cell-free products programed by the mRNA preparation was restricted to ovalbumin. Thus possible contamination by non-ovalbumin mRNAs could not be ruled out. Furthermore, fingerprint analysis of enzymic digest of the cell-free products (a crucial test for proving biological purity) was not done.

In a previous publication some properties (molecular weight, capacity to program the synthesis of precursor protein, etc.) of biologically pure mRNA coding for a mouse immunoglobulin light chain (L chain) were described (Schechter, 1973). It was prepared from MOPC-321 myeloma polysomes specifically precipitated with antibodies directed to L chains, followed by chemical purification on oligo-dT-cellulose. The main criterion employed for determining authenticity and biological purity (≥95%) was the fingerprint analysis of tryptic digest of the total reaction mixture containing cell-free products programed by the mRNA. Chemical purity (95%) was determined from the amount of rRNA in the mRNA preparation by scanning of appropriate gels. In the present report the double antibody technique used for the preparation of this mRNA is described.

Experimental Section

Materials. The MOPC-321 myeloma tumor (a generous gift of Dr. M. Potter) was maintained as a solid tumor in female Balb/c mice, and the L-chain protein that it secretes was isolated from the urine, as described (Potter, 1967). The MOPC-41 L-chain protein was a generous gift of Dr. E. Appella. [^{3}H]Leucine (35 Ci/mmol) and ^{14}C-labeled amino acids (90–320 Ci/mol) were obtained from New England Nuclear. Heparin-sodium in sterile saline (1000 units/ml, 1

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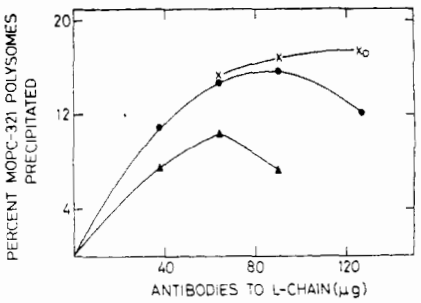

FIGURE 1: Precipitation curves of MOPC-321 polysomes as a function of antibodies concentrations. Constant amounts of MOPC-321 total polysome population (20 A_{260} units per tube) were reacted with increasing amounts of goat anti-L-chain antibodies, and then with rabbit anti-goat-Ig. See text for reaction conditions. Amounts of rabbit anti-goat-Ig added were: (▲) 0.6 mg; (●) 0.9 mg; (×) 1.2 mg; (○) 1.5 mg.

unit equivalent to about 0.01 mg of heparin) was purchased from the Upjohn Co., Kalamazoo, Mich. Glass-distilled water was used for preparing solutions, and all glassware was sterilized. Unless stated otherwise, all manipulations were carried out at +4°.

Preparation of Total Polysome Population. Seventeen to twenty days after inoculation into mice, the solid tumors were collected into liquid nitrogen and stored at −70° until used. In a typical preparation 80 g of tumor was thawed in 2.5 volumes of 0.88 M sucrose in solution C (50 mM Tris·HCl (pH 7.4)–25 mM NaCl–5 mM MgOAc–7 mM 2-mercaptoethanol) containing 10 units/ml of heparin, 0.6% sodium deoxycholate, and 0.6% Triton X-100. It was homogenized first in a Waring blender (4 spins, each of 10 sec) and then in a Dounce homogenizer (seven strokes with a loose pestle followed by one stroke with a tight-fitting pestle). The homogenate was centrifuged (20,000*g*/20 min), and aliquots of the supernatant (15 ml) were layered over a cushion (11 ml) of 1.5 M sucrose in solution C containing 5 units/ml of heparin, and spun (40,000 rpm/10 hr) at 2° in the Spinco 60-Ti rotor. Care was taken to remove the supernatant completely. The polysomal pellet was rinsed gently with 0.25 M sucrose in solution C containing 5 units/ml of heparin, then it was suspended in this medium and stored in liquid nitrogen until used. The yield of polysomes was 170–180 A_{260} units per gram of tumor. This yield was somewhat higher than that obtained in earlier experiments when tissue homogenization was done without detergents (130–150 of A_{260} units polysomes per g, see Schechter (1973)). Apparently, the detergents released polysomes from heavy microsomes that otherwise would have been removed by centrifugation of the homogenate at 20,000*g* for 20 min (Becker *et al.*, 1970). Polysomes prepared with solutions containing or lacking detergents were equally good as a source for mRNA preparation.

Preparation of mRNA from Total Polysome Population. The procedure used to isolate this mRNA preparation was described before (Schechter, 1973).

Analytical Immune-Precipitation of Polysomes. The reactants were prepared as follows. To an ice-cold polysome suspension (180–260 A_{260}/ml) were added in the following order: heparin (1000 units/ml), sodium deoxycholate (20%), and Triton X-100 (20%) to final concentrations of 10 units/ml, 0.4 and 0.4%, respectively. The suspension was spun in a chilled Servall centrifuge (9500*g*/10 min). The supernatant was collected with a pasteur pipet and saved. The gelatinous material that precipitated contained 5–11% of the A_{260} absorbing material and was discarded. Solutions containing antibodies or normal goat-Ig were supplemented with heparin, sodium deoxycholate, Triton X-100, and MgOAc to final concentrations of 10 units/ml, 0.4%, 0.4% and 5 mM, respectively, and then clarified by centrifugation (12,000*g*/20 min).

Equal aliquots of the clarified polysomal suspension (about 20 A_{260} units) were divided into test tubes. Increasing amounts of goat anti-L-chain antibodies (as well as normal goat-Ig) were added, and 6 min later increasing amounts of rabbit anti-goat-Ig were added. After 30 min the reaction mixture (final volume about 0.3 ml) was spun in a chilled International centrifuge (2200*g*/20 min), the precipitate was suspended in 0.7 ml of solution D (100 mM sucrose–25 mM Tris·HCl (pH 7.4)–50 mM KCl–5 mM MgOAc–, 0.2% Triton X-100–10 units/ml of heparin) and centrifuged as above. The precipitate was washed once more in solution D and dissolved in 0.1 N NaOH (1 or 2 ml) and the absorbancy at 260 and 280 nm was recorded. A series of tubes containing "antigen" (goat anti-L-chain, or normal goat-Ig) and antibody (rabbit anti-goat-Ig) without polysomes, and other controls were included in each experiment (see Figure 1 and Table I).

The amount of polysomes in the precipitate was calculated from the difference in absorbances of solutions a and b prepared from dissolved precipitates, where a was obtained from reaction mixtures containing "antigen," antibody, and polysomes; b was obtained from reaction mixtures containing the same amounts of "antigen" and antibody but no polysomes. These simple measurements were found to be satisfactory, since the ratio of the differences at 260 and 280 nm ($\Delta A_{260}/\Delta A_{280}$) was similar to the A_{260}/A_{280} value of the original polysome suspension (see Table I). Also, the amount of RNA extracted from immune-precipitated polysomes (on a large scale experiment) corresponded to the amount of polysomes precipitated in the analytical titration experiment (see Results).

Preparation of mRNA from Immune-Precipitated Polysomes. The amounts of antibodies employed in a large scale experiment were calculated from the results of the analytical experiment (see Figure 1). A typical experiment is described below. Before use the antibody solutions were supplemented with heparin (10 units/ml), sodium deoxycholate (0.4%), Triton X-100 (0.4%), and MgOAc (5 mM) and clarified by centrifugation (12,000*g*/20 min). MOPC-321 polysomes (43 ml containing 11,800 A_{260} units and A_{260}/A_{280} ratio of 1.74) in solution C were made 10 units/ml in heparin, 0.4% in sodium deoxycholate, and 0.4% in Triton X-100 and spun (9500*g*/20 min). To the supernatant (39 ml, 10,800 A_{260} units, A_{260}/A_{280} = 1.75) were added purified goat anti-L-chain antibodies (5.2 ml containing 63 mg of antibody) and 6 min later purified rabbit anti-goat-Ig (60 ml, 780 mg of antibody). After 30 min the reaction mixture was spun (8000*g*/10 min) and the supernatant (A_{260}/A_{280} = 1.57) was discarded. The precipitate that contained "antigen"–antibody complexes together with polysomes was suspended in solution D (100 ml) by repeated blowing from a 10-ml pipet, spun (8000*g*/10 min), and washed again in solution D. The RNA was then extracted from the washed precipitate with the aid of solution E (100 mM Tris·HCl (pH 9.0)–100 mM sucrose–10 mM KCl–2 mM MgOAc–3% sodium dodecyl sulfate) and phenol. Solution E (50 ml) was added at room temperature, and the precipitate was brought into a fine homogeneous suspension by fast repeated blowing from a 10-ml pipet. The suspension (containing 2340 A_{260} units, A_{260}/A_{280} = 1.69) was mixed with an equal volume of water-saturated phenol, and it was agitated gently at room temperature for 10 min. After cooling in ice for 5 min it was spun in a chilled Servall centrifuge (12,000*g*/10 min), the

TABLE 1: Analytical Immune Precipitation of Polysomes by the Double Antibody Technique.

	Tube No.[a]																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Reaction components[b]																					
MOPC-321 Polys.[c]						100	100	100	100	100	100	100	100	100	100	100					
Krebs II Polys.[d]																	150	150	150	150	150
Solution C supp.[e]	100	100	100	100	100						200	200	100	200	100					200	
Pu. Ab. to L chain[f]	10	10	10			10	10	10				10	110				10	10			
Normal goat-Ig[g]				5	12				5	12				5	100				5		
Pu. Ab. to goat-Ig[h]	100	150	200	100	200	100	150	200	100	200						200	100	200	100		200
Results																					
A_{260}[i]	0.40	0.60	0.73	0.65	1.42	1.94	3.81	4.16	1.03	1.97	0.14	0.14	0.36	0.14	0.13	0.18	0.78	1.09	1.04	0.08	0.11
A_{280}[i]	0.33	0.49	0.59	0.53	1.13	1.15	2.25	2.46	0.74	1.46	0.11	0.12	0.26	0.12	0.11	0.14	0.55	0.79	0.76	0.09	0.12
A_{260}/A_{280}	1.21	1.22	1.23	1.23	1.25	1.68	1.69	1.69	1.39	1.35	1.27	1.17	1.23	1.17	1.18	1.28	1.42	1.38	1.37	0.89	0.92
ΔA_{260}[j]						1.54	3.21	3.43	0.38	0.57			0.22[k]				0.38	0.36	0.39		
ΔA_{280}[j]						0.82	1.76	1.87	0.21	0.33			0.15[k]				0.22	0.20	0.23		
$\Delta A_{260}/\Delta A_{280}$						1.88	1.82	1.83	1.81	1.72			1.46				1.73	1.80	1.70		
% Polys. precip. from																					
ΔA_{260}[l]						7.6	15.8	16.9	1.9	2.8	0.7[m]		1.1				2.1	2.0	2.1	0.4[m]	
ΔA_{280}[l]						7.3	15.6	16.6	1.9	2.9	1.0		1.3				2.2	2.0	2.3	0.9	

[a] All reactions were carried out in duplicates, the absorbances of dissolved precipitates in a pair differed by up to 7%. [b] Numbers represents μl of reactant added. [c] Suspension of MOPC-321 total polysome population containing 203 A_{260} units/ml, A_{260}/A_{280} = 1.80 (measured in 0.1 N NaOH). [d] Krebs II ascites polysomes were prepared as MOPC-321 polysomes. The suspension contained 182 A_{260} units/ml, A_{260}/A_{280} = 1.83 (measured in 0.1 N NaOH). [e] Solution C supplemented with 0.25 M sucrose, 10 units/ml of heparin; 0.4% sodium deoxycholate, and 0.4% Triton X-100. [f] 9 mg/ml of purified goat anti-L-chain antibodies. [g] 10 mg/ml. [h] 6 mg/ml of purified rabbit anti-goat-Ig. [i] Absorbancy of immune precipitate dissolved in 1 ml of 0.1 N NaOH. [j] Difference of absorbances of solutions of reaction mixtures with and without polysomes but containing the same amounts of other reactants, except for tube 13. [k] Difference between values of tubes 13 and 11. [l] Per cent polysome precipitated from ΔA_{260} and ΔA_{280} values. It was calculated by dividing the latter values with the corresponding absorbances of the original polysome suspension and multiplication by 100, except for tubes 11 and 20. [m] Calculated from A_{260} and A_{280} values of the precipitate, and of the original polysome suspension.

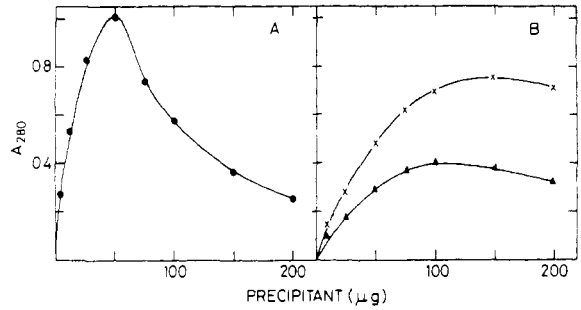

FIGURE 2: Quantitative precipitin curves of purified antibodies. Absorbances at 280 nm of immune precipitates dissolved in 1 ml of 0.1 N sodium hydroxide: (A) 1 mg of goat anti-L-chain (MOPC-321) reacting with (●) L chain (MOPC-321); (B) 0.6 mg of rabbit anti-goat-Ig reacting with (▲) purified goat anti-L-chain and (×) normal goat-Ig.

aqueous phase was separated and the phenol phase was re-extracted once at room temperature (10 min) with 40 ml of a solution containing 10 mM Tris·HCl (pH 9.0)–0.5% sodium dodecyl sulfate. Chilling and phase separation were done as above. The aqueous phases were combined and reextracted three times with phenol at +4°. The RNA was precipitated by adding 0.1 volume each of 2.0 M NaCl and of 2.0 M NaOAc (pH 5.5), and 2.5 volumes of ethanol at −20°. The amount of RNA obtained was 1950 A_{260} units (A_{260}/A_{280} = 1.96). The isolation of MOPC-321 L-chain mRNA from this RNA by means of sucrose gradient centrifugation and oligo-dT-cellulose were described before (Schechter, 1973).

Cell-Free Synthesis of Proteins. Translation of exogenous mRNA was carried out in the preincubated Krebs II ascites cell-free system (Mathews, 1970; Aviv *et al.*, 1971) that was modified as described (Schechter, 1973). The reaction products were labeled either with [^{3}H]leucine or with ten [^{14}C]amino acids (Ala, Arg, Gly, Ile, Leu, Lys, Pro, Ser, Thr, Val). Endogenous activity of myeloma polysomes was studied in the same system, except that the Krebs II polysomes were replaced by MOPC-321 polysomes that were purified of cell sap as described above.

Normal Goat-Ig. A protein fraction was precipitated from pooled normal goat sera with ammonium sulfate at 45% saturation at 5°, and then twice dissolved in water and reprecipitated as above. The precipitate was dissolved and dialyzed extensively in the phosphate-buffered saline (0.9% NaCl–0.01 M Na_3PO_4 buffer (pH 7.4)).

Immunization Procedure. Normal goat-Ig was injected into rabbits and MOPC-321 L chains into goats. Hyperimmune sera were obtained by injecting the antigens (2 mg per injection) in complete Freund's adjuvant, as described (Schechter *et al.*, 1966).

Immunoadsorbents. The proteins were coupled to Sepharose 4B (Pharmacia, Uppsala) *via* cyanogen bromide according to published procedures (Cuatrecasas, 1970). The final products contained: 350 mg of normal goat-Ig bound to 25 g (wet weight) of Sepharose; 60 mg of MOPC-321 or MOPC-41 L chains bound to 15 g (wet weight) of Sepharose. The first immunoadsorbent could remove from appropriate rabbit antisera 900 mg of purified antibodies directed toward goat-Ig; the latter immunoadsorbents could remove from goat antisera 300 mg of anti-L-chain antibodies.

Preparation of Purified Antibodies. The same purification procedure was applied for all antisera. The antiserum was spun 16,000*g*/15 min to remove fat and a small amount of precipitate. The immunoadsorbent was packed in a sterile syringe. The clarified antiserum was passed through this column at room temperature at a rate of about 100 ml/hr, and the column was then washed with phosphate-buffered saline. With L-chain immunoadsorbents the absorbancy of the eluate dropped to 0.02–0.04 A_{280} unit after 300 ml, and washing was continued up to 500 ml of phosphate-buffered saline. With goat-Ig immunoadsorbent the absorbancy of the eluate dropped to about 0.1 A_{280} unit after 400 ml and did not change by continued washing up to 800 ml of phosphate-buffered saline, which was apparently due to small amounts of antibody (or antigen–antibody complexes) that leaked from the column. The adsorbed antibody was eluted from the column by washing with 0.1 M acetic acid. The acidic eluate was monitored at 280 nm. Fractions containing the protein peak were pooled, neutralized with 0.5 equiv of NH_4OH, and dialyzed at 4° against phosphate-buffered saline (4 l. × 3) for 2 days. A small amount of precipitate was removed by centrifugation, and the dialyzed pure antibody solution was stored at −20°.

From the quantitative precipitin curves (Figure 2) it was calculated (Schechter *et al.*, 1971c) that MOPC-321 L chains precipitated 65% of the pure goat anti-L-chain antibodies. From rabbit anti-goat-Ig, 60% of the purified antibodies were precipitated by normal goat-Ig, 30% by pure goat anti-L-chain antibodies. The difference in precipitation potency of the two antigens might be attributed to the heterogenous nature of the goat-Ig preparation used for immunization. This preparation (45% ammonium sulfate cut) contained several Ig classes and other serum proteins, whereas, the purified goat anti-L-chain antibody was mainly composed of IgG (Schechter *et al.*, 1971c) since it was prepared from hyperimmune sera.

Conversion values: 1.4 A_{280} units for 1 mg of Ig; 13 A_{260} units for 1 mg of polysomes; 20 A_{260} units for 1 mg of RNA.

Results

The specific isolation of polysomes involved in the synthesis of a particular protein was achieved by the double antibody technique. In the first stage antibodies to L chains were allowed to react with polysomes containing nascent L chains to form a soluble antibody–polysome complex. In the second stage another antibody with specificity directed toward the Ig moiety in the complex was added, resulting in the formation of aggretates that precipitated out of solution.

Analytical Immune-Precipitation of Polysomes. This experiment was done on a small scale in order to determine the optimal amounts of antibodies required to achieve maximal specific precipitation of polysomes. For maximal precipitation it was necessary: (1) to saturate all polysomes carrying nascent L chains with goat antibodies to L chain; (2) to achieve quantitative precipitation of the above antibody–polysome complex with the aid of rabbit anti-goat-Ig.

The extent of polysome precipitation as a function of either antibody concentration is summarized in Figure 1. A portion of the protocol of this experiment is given in detail in Table I; it contains some of the crude data that served for plotting Figure 1 as well as controls bearing on the specificity of the reaction. The titration curves (Figure 1) at constant anti-goat-Ig (0.6 or 0.9 mg of antibodies) showed that 36 μg of anti-L-chain antibodies did not saturate all relevant polysomes because an increase to 63 μg was accompanied with an increase in polysome precipitation. A further increase to 90 μg gave varying results depending upon the amount of rabbit anti-goat-Ig. At low anti-goat-Ig contents (0.6 mg) a decrease in yield was observed (this is analogous to the region of antigen excess, here displayed by anti-L-chain antibodies, in a regular

precipitin curve, see Figure 2). However, at 90 μg of anti-L-chain antibodies, polysome yields increased with increasing anti-goat-Ig from 0.6 to 0.9 mg, and the yield tended to level off with a further increase to 1.2 mg. From the above examples the optimal conditions for maximal polysome precipitation were defined: (1) no increase or decrease in polysome yield with an increase in goat anti-L-chain antibodies; (2) no increase in polysome yield with an increase in anti-goat-Ig. By applying these guidelines to the data in Figure 1, it could be seen that for 20 A_{260} units of polysomes the optimal amounts of goat anti-L-chain and of anti-goat-Ig were about 110 μg and 1.5 mg, respectively. It is necessary to make titration curves for every batch of antibodies because they might have different affinities. That is, using the same polysome and goat anti-L-chain solutions as in Figure 1, but a different anti-goat-Ig preparation, the optimal amounts of anti-goat-Ig and goat anti-L-chain gave a ratio (w/w) of about 20 while in Figure 1 this ratio was about 14 (1.5/0.11).

When the reaction mixtures were kept at +4° for 20 hr instead of 30 min (after addition of anti-goat-Ig), it was found that the amount of precipitate in the controls (tubes 2–4, Table I) was not changed, but in the corresponding experimental tubes (tubes 7–9, Table I) the calculated amount of precipitated polysomes was reduced to about 70%. Therefore, the shorter reaction time was adopted for polysome immune precipitation.

The specificity of the immune-precipitation reaction is shown below. From the total MOPC-321 polysome population the fractions precipitated were: 17.3% with goat anti-L-chain (see Figure 1) and 1.9% with normal goat-Ig (tube 9, Table I). In both cases similar amounts of "antigen"–antibody precipitates were formed by the addition of rabbit anti-goat-Ig (tubes 3 and 4, Table I). Without any antibody added, 0.7% of the polysomes precipitated (tube 11, Table I). Thus, the observed specificity ratio for the precipitation reaction was 14 ([17.3 − 0.7]/[1.9 − 0.7]). However, with respect to mRNA purification, this ratio would increase if we assume that about 30% of the material that coprecipitated with unrelated antibodies did not carry functional mRNA (single ribosomes and oligosomes with defective mRNA). Then the corrected specificity ratio would be 20 ([17.3 − 0.7]/[1.9 − 0.7]0.7). In addition, when Krebs II ascites polysomes were similarly treated with the two "antigen"–antibody systems (tubes 17–19, Table I), equal and low amounts of polysomes were precipitated (2.0 and 2.1%).

The extent of nonspecific precipitation of polysomes increased from 1.9 to 2.8% with increasing amounts of "antigen"–antibody precipitates (see tubes 9 and 4 and 10 and 5 in Table I). Nevertheless, the analytical experiment defined the minimal amounts of specific antibodies (*i.e.*, of precipitates) required for maximal polysome precipitation.

The one-stage precipitation procedure with anti-L-chain antibodies alone was inefficient for polysome precipitation (compare tube 12 with tubes 6–8, Table I) even when the amount of anti-L-chain antibodies was increased by a factor of 11 (see tubes 13, 12, and 6–8, Table I). Also the addition of MOPC-321 L chains together with excess of anti-L-chain antibodies did not give satisfactory results, only 1–1.5% of the polysomes were precipitated. This was true when the source of anti-L-chain antibodies was purified antibodies, Ig isolated from antiserum to L chain (45% ammonium sulfate cut), or the $(Fab_2)'$ prepared from this Ig by pepsin digestion (unpublished data).

Preparation of mRNA from Immune-Precipitated Polysomes. In the process of polysome isolation the following points are worth mentioning. (1) The immune precipitate containing polysomes was washed twice with solution D, and the first and second supernatants had A_{260}/A_{280} ratios of 0.89 and 0.68, respectively. These low values indicate that the polysomes were effectively trapped in the precipitate. (2) To prevent nuclease activity sodium dodecyl sulfate was included in solution E that was used to suspend the immune precipitate. The volume of solution E was such that its sodium dodecyl sulfate contents was 3–4-fold (w/w) the amount of protein in the precipitate. The latter was estimated from the analytical experiment, taking into account protein in the form of "antigen"–antibody complex, and half of the mass of precipitated polysomes as protein. (3) The amount of RNA extracted from the immune-precipitated polysomes (1950 A_{260} units of RNA from a total of 10,800 A_{260} units of polysomes, *i.e.*, about 18% yield) was in good agreement with the amount expected from the analytical experiment (17.3% yield, from Figure 1).

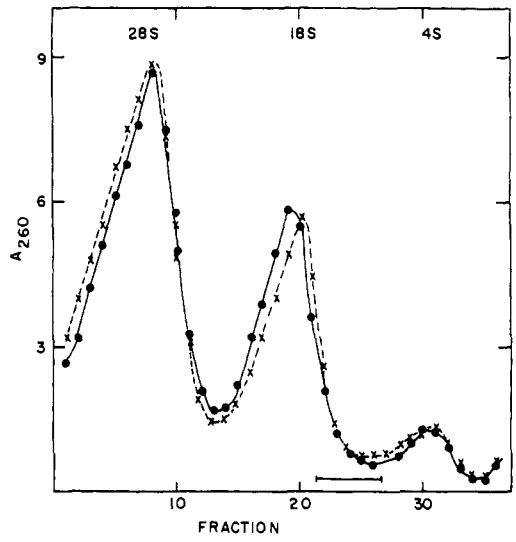

FIGURE 3: Sedimentation profile of RNA extracted from MOPC-321 myeloma polysomes. Each RNA sample was layered over a 15–30% sucrose gradient made in solution B (10 mM Tris·HCl (pH 7.4)–100 mM NaCl–1 mM Na_2EDTA–0.4% sodium dodecyl sulfate) and spun 26,000 rpm for 14 hr at 22° in a Spinco SW-27 rotor. Polysome source: (●) total population; (×) immune precipitated (using either anti-L-chain, anti-C, or anti-V antibodies). Horizontal bar indicates the 12S fraction pooled.

The L-chain mRNA was isolated from the RNA extracted from the immune-precipitated polysomes in two stages. The RNA was run on a sucrose gradient, and the sedimentation profile obtained was indistinguishable from that of RNA extracted from regular polysomes (Figure 3). The fraction sedimenting at about 12 S was pooled, and in a cell-free system it was fairly active in directing the synthesis of the L-chain protein (Schechter, 1973). This crude fraction was further purified from rRNA by passage on oligo-dT-cellulose[1] (Figure 4). It should be noted that the chemical purification from rRNA caused an increase in specific activity, but it did not affect biological purity. That is, mRNA preparations before and after passage on oligo-dT-cellulose directed in a cell-free system the synthesis of products that had the same pattern of mobilities in sodium dodecyl sulfate polyacrylamide gels, and that gave the same fingerprints (Schechter, 1973).

[1] Equally good results were obtained when the RNA extracted from immune-precipitated polysomes was applied directly to oligo-dT-cellulose column without prior fractionation by sucrose gradient centrifugation (unpublished data).

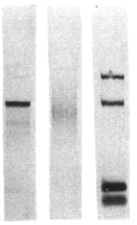

FIGURE 4: Gel electrophoresis of L-chain mRNA preparations. Samples were analyzed on a slab gel containing 1.7% acrylamide–0.5% agarose at 250 V for 1.5 hr (Peacock and Dingman, 1968). The samples are: (1) the crude 12S RNA fraction from immune-precipitated polysomes (see Figure 3); (2) same as 1 but after purification on oligo-dT-cellulose that was done essentially as described (Edmonds and Caramela, 1969); (3) 28S, 18S, 5S, and 4S RNA markers from MOPC 321 ribosomes.

mRNA from Polysomes Precipitated with Antibodies Specific to the N- and C-Terminal Regions of the L Chain. Protein biosynthesis on the ribosome starts from the N-terminal end of the polypeptide chain, and there is one initiation site on the mRNA for the entry of ribosomes to form a polysome. Therefore, it is expected that of the total mass of nascent chains growing from polysomes, the fraction of N-terminal regions would be larger and more accessible for interaction with the surrounding than the fraction of C-terminal regions. It was now of interest to find out whether antibodies directed toward the N-terminal portion of the protein would precipitate more polysomes than antibodies directed toward its C-terminal portion. A preliminary experiment designed to test this possibility is described below.

The MOPC-321 and MOPC-41 L chains are of the κ-type proteins (McKean *et al.*, 1973b). They share an identical constant region (C region) which is the C-terminal half of the molecule, but they differ at the variable region (V region) which is the N-terminal half. From the known amino acid sequence of MOPC-321 (McKean *et al.*, 1973a) and of MOPC-41 (Gray *et al.*, 1967) it can be seen that the V regions of MOPC-321 (111 residues) and of MOPC-41 differ in 53 positions. These structural differences are reflected in the cross-precipitation reactions shown in Figure 5A. In a first approximation it could be assumed that in the total antibody population elicited by injecting MOPC-321 L chains, the antibodies that reacted with MOPC-41 L chain were mainly specific for the C region (designated as anti-C), and the rest were mainly

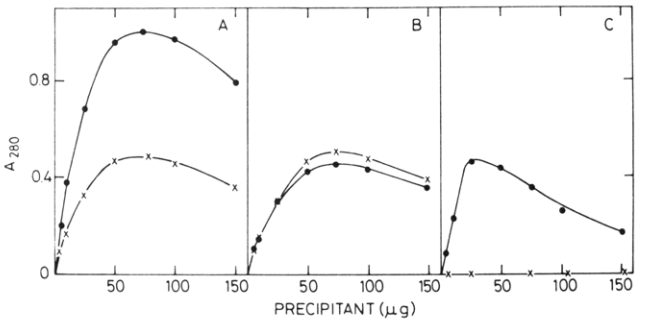

FIGURE 5: Cross-precipitation reactions of antibodies to MOPC-321 L chain. Absorbances at 280 nm of immune precipitates dissolved in 1 ml of 0.1 N sodium hydroxide. The antibodies are in: (A) 0.3 ml of goat antiserum to intact MOPC-321 L chain; (B) 0.7 mg of purified anti-C antibodies with specificity directed mainly toward the constant region of MOPC-321 L chain; (C) 0.7 mg of purified anti-V antibodies with specificity directed mainly toward the variable region of MOPC-321 L chain. The precipitants are (●) MOPC-321 L chain and (×) MOPC-41 L-chain.

TABLE II: Summary of Analytical Immune-Precipitation Experiments Using Anti-C and Anti-V Antibodies.[a]

Antibodies Added	Per Cent Polysomes Precipitated from: Total Population	Polysomes Previously Depleted with: Anti-C	Anti-V
Anti-L-chain + anti-goat-Ig	17.3	8.1	5.7
Anti-C + anti-goat-Ig	13.8	6.1	5.7
Anti-V + anti-goat-Ig	17.6	7.4	5.0
Normal-goat-Ig + anti-goat-Ig	1.8	3.2	3.3
Anti-C	0.7	0.8	0.9
Anti-V	0.7	0.8	0.9
Anti-goat-Ig	0.8	1.0	1.1
None	0.7	0.8	0.8

[a] The maximal amounts of polysomes precipitated in these experiments are given in the table. Anti-L-chain are purified goat antibodies to MOPC-321 L chain. Anti-C and anti-V are purified goat antibodies with specificity directed toward the constant and variable regions, respectively, of the L chain.

directed to the V region of MOPC-321 L chain (designated as anti-V). The antibodies with anti-C and anti-V specificities were separated by passing the antiserum first on Sepharose-MOPC-41 L-chain conjugate that retained the anti-C. The partially adsorbed antiserum was then passed over Sepharose-MOPC-321 L chain conjugate that retained the anti-V. The precipitin reactions of the purified antibodies released from the immunoadsorbents appeared to confirm their tentative designation (Figure 5B and C). The anti-C reacted equally well with MOPC-321 and MOPC-41 L chains (in contrast to the original antiserum where MOPC-41 L chain showed only 44% cross-reaction). The anti-V did not show any cross-precipitation reaction with MOPC-41 L chain.

Analytical immune-precipitation experiments were carried out, using anti-C and anti-V antibodies. The maximal amounts of polysomes precipitated are given in the second column of Table II. It is seen that the total antibody population (anti-L-chain) and the fraction directed mainly toward the N-terminal half of the L chain (anti-V) were equally good (17.3 and 17.6% polysomes precipitated). The antibody fraction directed mainly toward the C-terminal half of the L chain (anti-C) was somewhat less efficient (13.8%); it had 80% of the polysome-precipitating capacity as compared to the above antibodies.

In the next step large scale precipitation of polysomes was performed with the antibody fractions, the RNA extracted from the immune-precipitated polysomes was run on a 15–30% sucrose gradient, the 12S region was separated (Figure 3), and its mRNA activity was assayed in the Krebs II cell-free system. In terms of stimulating the incorporation of [^{3}H]leucine into Cl_3CCOOH precipitable material, similar activities were found in the 12S RNA fractions prepared from polysomes precipitated with the aid of anti-L-chain, anti-V, and anti-C antibodies (Table III). The molecular weight distribution of the cell-free products in sodium dodecyl sulfate polyacrylamide gels was also similar in all three cases (Figure 6).

mRNA from Polysome Exposed Twice to Antibody Precipitation. Because anti-C antibodies precipitated 80% of the

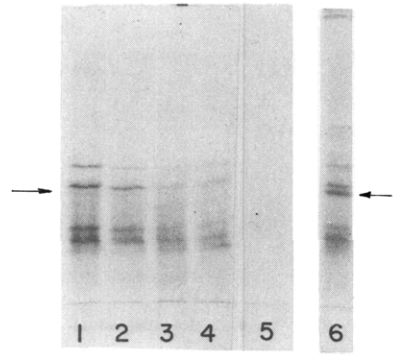

FIGURE 6: Autoradiogram of sodium dodecyl sulfate polyacrylamide gels of cell-free products. Total reaction mixtures labeled with ten [^{14}C]amino acids were reduced and analyzed on slab gels (13% acrylamide, 16 V/cm, 1.5 hr) essentially as described (Maizel, 1972). The products were programed by the 12S RNA fraction extracted from polysomes immune precipitated with: (1) anti-L-chain; (2) anti-V; (3) anti-C_1V_2; (4) anti-C; (5) minus mRNA (control); (6) (run made on a separate gel) endogenous activity of MOPC-321 polysomes. Arrows indicate the position of MOPC-321 L-chain marker applied with the cell-free sample. Molecular weight standards were ovalbumin, glyceraldehyde phosphate dehydrogenase, MOPC-321 L-chain, myoglobin, and hemoglobin.

MOPC-321 L chain bearing polysomes, an attempt was made to isolate and prepare mRNA from the residual 20%. Polysomes previously treated with anti-V antibodies were also analyzed.

The immune-precipitation reaction mixtures that contained the total MOPC-321 polysome population together with anti-C (or anti-V) and anti-goat-Ig antibodies were spun. The immune precipitates were used to prepare the mRNAs described in the previous section, and the supernatants (A_{260}/A_{280} = 1.55) were saved for further treatment. The polysomes in these supernatants were separated from residual antibodies by centrifugation (40,000 rpm/10 hr in the Spinco 60-Ti rotor at 2°) over a cushion of 1.5 M sucrose in solution C containing 5 units/ml of heparin. The supernatants over the sucrose cushion had A_{260}/A_{280} values of 0.51 and were discarded. The polysomal pellets were suspended in 0.25 M sucrose in solution C containing 5 units/ml of heparin. These suspensions had A_{260}/A_{280} values of 1.83. The extent of polysome precipitation by various antibodies in analytical experiments is summarized in Table II (third and fourth columns). The controls in which none or only one antibody was added remained low (0.8–1.1% precipitation). The control of normal goat-Ig plus anti-goat-Ig had increased from 1.8% in untreated polysomes to 3.2 and 3.3% in polysomes previously exposed to antibody. In the presence of two antibodies the yields were low as compared to untreated polysomes, but still higher than the control of normal goat-Ig plus anti-goat-Ig. Despite the low values, it appeared that the net amount precipitated from polysomes previously depleted with anti-C was somewhat higher than the net amount precipitated from polysomes previously depleted with anti-V; also, in the polysomes previously depleted with anti-C further treatment with anti-L-chain or anti-V yielded more polysomes than treatment with anti-C, as expected.

In the next step, large scale immune precipitation was carried out. Polysomes previously depleted with anti-C antibodies were now reacted with anti-V (to yield anti-C_1V_2 immune-precipitated polysomes), and *vice versa*. The RNAs were extracted from the precipitates, loaded on 15–30% sucrose gradients, and the 12S fractions were collected and tested for mRNA activity. The first relevant finding was that the RNA profile in the sucrose gradient centrifugation was indistinguishable from the profile of RNA extracted from regular polysomes, indicating that there was no massive degradation of rRNA upon double exposure to antibodies. In the Krebs II cell-free system mRNA activity was higher in the 12S fraction from anti-C_1V_2 treated polysomes than in the 12S fraction from anti-V_1C_2 polysomes (Table III). The molecular weight distribution of the products directed by the 12S RNA from anti-C_1V_2 was indistinguishable from the usual pattern (Figure 6). A similar pattern was also observed in the autoradiogram of products directed by the 12S RNA from anti-V_1C_2 polysomes, but the bands were too faint for photography.

TABLE III: mRNA Activity in the 12S Fraction of RNA Extracted from Polysomes Precipitated with Various Antibodies.[a]

Antibody Specificity	pmoles of Leu Incorporated per 0.01 A_{260} per 1.5 hr
Anti-L-chain[b]	0.70
Anti-C[b]	0.65
Anti-V[b]	0.74
Anti-C_1V_2[c]	0.55
Anti-V_1C_2[d]	0.33

[a] The polysomes were reacted with the purified antibodies listed in the table, and then precipitated with rabbit anti-goat-Ig. The RNA extracted from the immune precipitate was run on a sucrose gradient; the 12S region was isolated (see Figure 3) and assayed in the Krebs II cell-free system. Numbers represent net incorporation, obtained by substracting 0.25 pmol for the minus mRNA control. [b] Immune precipitates from total polysome population served for RNA extraction. [c] RNA extracted from immune precipitates obtained by reacting anti-V with polysomes previously depleted with anti-C antibodies. [d] RNA extracted from immune precipitates obtained by reacting anti-C with polysomes previously depleted with anti-V antibodies.

Discussion

Before discussing features of the polysome immune-precipitation procedure, previous results (Schechter, 1973) demonstrating the efficacy of antibody purification will be briefly summarized. The biological purity of the L-chain mRNA extracted from the immune-precipitated polysomes was calculated to be ≥95%. This value was based on the estimation of contamination by non-L-chain mRNA activities that were present in large abundance in RNA preparations extracted from the total polysome population. In Figure 7 it is seen that the fingerprint of products directed by mRNA extracted from the total polysome population contained many peptides: some of these could be identified as L-chain peptides, others were of non-L-chain origin. The fingerprint of products programed by mRNA from immune-precipitated polysomes was quite different. It contained 31 peptides. Twenty-seven of these were identical with authentic L-chain peptides (L chain yielded 28 peptides, Figure 7, panel 2). Since it was shown that this mRNA programed the synthesis of a L-chain precursor in which 20 amino acid residues were coupled to the N-terminal end of the mature L chain, it was suggested that the apparent loss of one peptide was due to a modified N terminus, and that the four extra peptides were derived from the extra

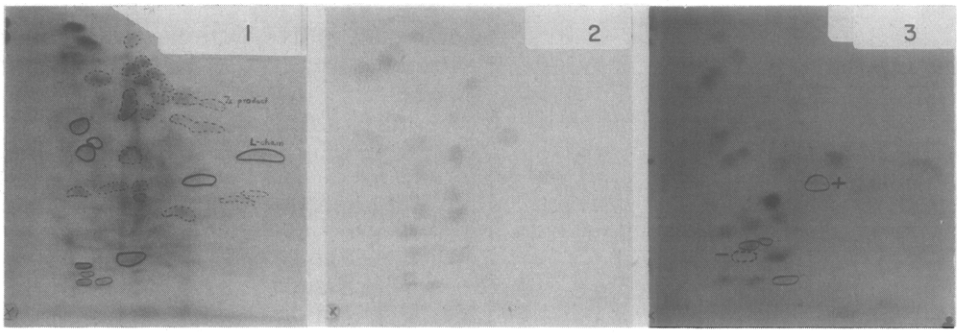

FIGURE 7: Fingerprints of cell-free products and of authentic MOPC-321 L chain. Autoradiograms of fingerprints of cell-free products, labeled with ten [^{14}C]amino acids, that were programed by 12S RNA fractions prepared from: (1) total polysome population; (3) immune-precipitated polysomes. Panel 2, ninhydrin stained fingerprint of cold MOPC-321. Total reaction mixtures kept at 36° for 4 hr and supplemented with 2 mg of MOPC-321 L chain were aminoethylated (Raftery and Cole, 1963), digested with L-1-tosylamido-2-phenylethyl chloromethyl ketone tryspsin (E:S ratio of 1:65), loaded on a Whatman 3 paper and subjected to chromatography (1-butanol–acetic acid–water, 17 hr) followed by electrophoresis at pH 3.5 (3000 V/50 min). After exposure to X-ray film the paper was stained with ninhydrin. Spots in autoradiogram and stained paper were matched. In panel 1, spots encircled with a continuous line were identified as peptides of L-chain origin (spots encircled with a dashed line matched with spots of non-L-chain peptides present in the fingerprint of cell-free products directed by the 7S RNA fraction extracted from total polysome population, the corresponding 7S region from RNA extracted from immune-precipitated polysomes had no detectable mRNA activity (Schechter, 1973)). In panel 3, peptides that are not seen here but detected in the original X-ray film are located in the middle left (2) and upper center (2) regions of the autoradiogram. These four matched with peptides in panel 2; peptides encircled with a continuous line are not detected in panel 2 and they probably originate from the extra piece in the precursor; the region encircled with the dashed line is empty here but occupied by a peptide in panel 2.

piece. The L-chain mRNA (sedimentation constant, about 15.5 S) had a tendency for aggregate formation, yet aggregates of various sizes (up to 35 S) had the same biological purity. By summing up the data related to the various L-chain mRNA fractions obtained from the sucrose gradient (see Table I in Schechter (1973)) it could be shown that 2% of the RNA extracted from immune-precipitated polysomes was retained on oligo-dT-cellulose. By correcting for the various degrees of contamination with rRNA in these oligo-dT-cellulose purified fractions, it was found that 1% of the RNA obtained from immune-precipitated polysomes was chemically pure L-chain mRNA. (It seems that the amount of L-chain mRNA in the immune-precipitated polysomes was even higher because considerable amount of mRNA activity was found in the bulk of RNA that was not retained on the oligo-dT-cellulose, unpublished data.) Thus, the mRNA obtained with the aid of antibodies and oligo-dT-cellulose purifications was within the range of expected theoretical yield. In view of the fact that polysomes (25,000 A_{260} units) from 150 g of tumor tissue could be processed in one batch, the yield of polysomes in the immune precipitate (18%), and the yield of mRNA from the latter (1%), it is evident that biologically and chemically pure L-chain mRNA can be prepared in milligram amounts. The biological purity estimated from the activity of the mRNA in a cell-free system ($\geq$95%) is in good agreement with the specificity ratio (1:20) determined from the extent of precipitation of myeloma and non-myeloma polysomes, each with antibodies to L-chain and non-L-chain proteins (see Results in present report). These results show that the antibody procedure employed fulfilled two basic requirements: (1) the specific precipitation only of polysomes carrying nascent chains of the protein in question; (2) the reaction conditions are such that the mRNA attached to the immune-precipitated polysomes retained its biological activity.

Polysomes engaged in the synthesis of different proteins may be bound to the endoplasmic reticulum, and therefore a microsome particle may contain different mRNA species. Consequently, if microsomes were used, heterogeneous mRNA preparations would be obtained even under the appropriate conditions of specific binding of the antibodies to one type of nascent chains. To avoid this complication, detergents were used to prepare polysomes that served as the starting material for the mRNA preparation. In addition, it has been shown before that detergents reduce the nonspecific trapping, presumably of polysomes (Stavnezer and Huang, 1971) and of proteins (Palmiter *et al.*, 1971) on immune precipitates formed in tissue homogenates.

The immune-precipitation reaction was carried out on polysomes freed of cell sap. Had this reaction been performed on the cell homogenate the amounts of purified antibodies required in this system would have to be increased by more than 15-fold because of the free L chain in the cells. Antibodies to L chains should bind better to intact L chains that retain their native conformation than to nascent L chains on polysomes (Sela *et al.*, 1967). One gram of tumor tissue of MOPC-321 was found to contain about 5 mg of L chains (unpublished data). A *minimum* of 15 mg of anti-L-chain antibodies is required to bind 5 mg of L chains. This is based on the molecular weights of the reactants (150,000 and 24,020), the bivalency of the antibody, and the assumption that all the combining sites of antibodies are saturated with the antigen (from Figure 2 it is seen that 100 mg of antibody are required to precipitate 5 mg of L chains). On the other hand, only 1 mg of anti-L-chain antibodies was required for saturating the nascent L chains on polysomes obtained from 1 g of tumor tissue. This is based on polysome yields (170–180 A_{260} units/g) and the data in Figure 1. Thus, in terms of antibodies economy, and to avoid the unnecessary increase in the amount of "antigen"–antibody precipitate (this may increase the nonspecific precipitation of polysomes, see Results) it would be desirable to purify the polysomes from cell sap. The fact that none or a negligible amount of protein precipitate was formed by adding anti-L-chain antibodies to polysome suspension (tubes 11–13, Table I) show that in the process of polysome preparation (see removal of supernatant and rinsing of pellet in the Experimental Section) more than 99.5% of the L chain in the cell sap was removed.

In order to prevent degradation of the mRNA the antibodies should be freed of RNase activity (Palacios *et al.*, 1972; Uenoyama and Ono, 1972). Palacios *et al.* (1972) used as a source of antibody the protein fraction that precipitated from immune sera at 40% ammonium sulfate saturation. The Ig

preparation obtained was freed of RNase by chromatography on DEAE- and CM-cellulose and Sephadex G-100. In the present work purified antibodies were used. These were prepared by immunospecific adsorption on insoluble matrix, and before elution the immobilized antibodies were washed with phosphate-buffered saline. Although specific assays for RNase were not performed, the data quoted below indicate that these preparations were practically free of RNase activity. Animal sera contain large amounts of hydrolytic enzymes. Earlier studies from our laboratory showed that the strong proteolytic activity present in antisera (Schechter *et al.*, 1966) was no longer detectable in purified antibodies isolated from the antisera (Schechter *et al.*, 1971c). In the present work the profile of RNA extracted from polysomes that had been kept with antisera showed signs of extensive degradation: the 28S and 18S rRNA peaks were of comparable size, the trough between the peaks was very high, etc. (data not shown). On the other hand, the profiles of RNAs extracted from immune-precipitated or regular polysomes were indistinguishable (Figure 3). The same was true for RNA extracted from polysomes exposed twice to the immune-precipitation procedure (see Results). Apparently not only the rRNA was kept intact, but the L-chain mRNA was not degraded either by antibodies, as suggested from the following data. If the antibodies had RNase activity, then the mRNA from polysome exposed twice to antibodies would be more degraded, and consequently it would be expected to direct the synthesis of low molecular weight products. Accordingly, the activities of mRNAs extracted from polysomes exposed to one or two cycles of antibody purification were compared. It was found that the cell-free products that they directed had similar patterns of molecular weight distribution (Figure 6). Further evidence supporting this point was the finding that the major protein bands formed by the endogenous activity of myeloma polysomes corresponded to the mRNA-dependent products (Figure 6). In the previous paper it was suggested that the wide range of molecular weights (28,700–17,200, see Figure 6) of the cell-free products, all of which were found to be L-chain polypeptides, was due to partial degradation of the mRNA. The latter was detected by polyacrylamide gel analyses of the mRNA (Schechter, 1973). In view of the results discussed above, it seems that this degradation occurred prior to contact with the antibodies, most probably during polysome isolation. Additional measures to prevent nucleases activity were the inclusion of heparin in the media used for polysome isolation, and of sodium dodecyl sulfate in solution E that was used to suspend the immune-precipitated polysomes.

Four general procedures (to be used separately or in combination), are suggested for the purification of the mRNA from the total RNA extracted from immune-precipitated polysomes. (1) Chromatography on insoluble carriers containing polyuridine or polythymidine, as well as on pure cellulose (see Schutz *et al.*, 1972). This procedure is based on the fact that most mRNAs of eukaryotes contain polyadenine sequences (Darnell *et al.*, 1971). (2) Chromatography on benzoylated-DEAE-cellulose. The basis of the separation on this column of mRNA and rRNA is not clear. This procedure was first used in bacteria (Sedat *et al.*, 1969), and recently it was used for the isolation of mRNA from HeLa cells (Murphy and Attardi, 1973). (3) Velocity centrifugation. After chromatography on the above columns the mRNA preparation may contain small but significant amounts of rRNA (Aviv and Leder, 1972; Schechter, 1973). Small amounts of rRNA can be effectively separated from the mRNA by sucrose gradient centrifugation, provided that they have different S values. (4) Gel electrophoresis in aqueous (Peacock and Dingman, 1968) and in dissociating media (Staynov *et al.*, 1972). This procedure has a high resolving power, but in contrast to the previous procedures it can be applied only to small amounts of material.

The specificities of anti-C and anti-V antibodies were mainly directed against different regions of the L chain (Figure 5). The findings that these antibodies precipitated polysomes in high yields (100 and 80%, respectively, of the maximum) indicate that antibodies to a particular antigen would be useful for isolating polysomes carrying nascent chains of cross-reacting antigens. This means that the biosynthesis of precursor proteins which contain extra pieces, even at the N terminus of the nascent chains, should not necessarily prevent the isolation of the relevant polysomes with the aid of antibodies to the mature protein. Indeed, it was shown that antibodies to L chain precipitated polysomes containing mRNA to a precursor of L chain. In this precursor an extra run of 20 amino acid residues were coupled to the N-terminal residue of the mature L chain (Schechter, 1973). Another implication to this situation has to do with the economy of reagents preparation, *e.g.*, it is predicted that with the aid of antibodies to a particular κ chain it would be possible to prepare mRNAs coding for most (if not all) κ chains. On the other hand, special precautionary measures would be required for isolating a particular mRNA from cells that synthesize several proteins which share common antigenic determinants. In this case it would be necessary to prepare antibodies restricted to the protein in question by adsorption with the cross-reacting proteins.

The analytical immune-precipitation procedure described here may serve as a simple and fast assay for determining the fraction of polysomes engaged in the synthesis of a particular protein. This information might be useful, *e.g.*, for studying cell differentiation (Palacios *et al.*, 1972). Our data, however, did not establish whether the antibodies precipitated all polysomes carrying nascent L chains. Nevertheless, it seems that most of the polysomes were precipitated, because from polysomes previously depleted with anti-V antibodies only 2.4% (5.7 − 3.3, see Table II) could be specifically precipitated. One approach to solve this question is to determine whether L-chain mRNA is still present in polysomes from the supernatant of immune precipitate. It would be possible to do this by hybridization experiments with complementary DNA to the mRNA to be synthesized with reverse transcriptase.

The first stage in polysome immune precipitation is the binding of antibodies to the incomplete nascent chains. Because incomplete proteins may not have the conformation of the intact protein (Taniuchi and Anfinsen, 1969) and because of the important role of the conformation of the antigen in defining the specificity of antibodies (Sela *et al.*, 1967), the general occurrence of antibody–nascent chain interaction could be questioned. The complete lack of cross-reaction between antibodies to the native protein with the denatured protein involved extensive modification of the latter (*e.g.*, complete reduction and alkylation, see Sela *et al.*, 1967). But this is not a general rule since there are examples where proteins that underwent considerable chemical modifications could still precipitate antibodies to the native protein (Omenn *et al.*, 1970a). It should be noted, however, that in the double antibody technique the requirement is that the first antibody will be bound to the nascent chain, rather than cause precipitation. In fact, in all cases investigated so far fragments of the intact antigen (provided that they were

not too small or chemically modified) were always bound by antibodies elicited toward the intact antigen. This may be due to several reasons. The fragments were large enough to retain portions of the conformation present in the immunizing antigen (Porter, 1957). Antibodies can be easily elicited against sequential determinants that are not dependent on the conformation of the intact antigen. This is true not only for haptens, but antibodies to fibrillar (Cebra, 1961) and globular (Benjamini *et al.*, 1964) proteins were also capable of reacting with short peptides derived from the parent protein. In addition it has been shown in proteins (Crumpton and Wilkinson, 1965; Omenn *et al.*, 1970b) and in synthetic antigens (Schechter *et al.*, 1971b) that antibodies to conformational determinants can bind fragments of the antigen which lack the appropriate conformation when free in solution. To explain these results it was suggested that the antibodies could induce the native conformation in the fragment. Indeed, it was shown that ligands changed conformation when bound at the antibody combining site (Crumpton, 1966; Schechter *et al.*, 1971a). The observation that the enzymic activity of enzyme nascent chains bound to ribosomes was increased by exposure to antibodies to the native enzyme (Cowie *et al.*, 1961; Hartlief and Koningsberger, 1968) seem to be a good example of induced conformation in antibody–nascent chain interaction.

The experiments described here confirm previous reports that polysomes could not be appreciably precipitated with antibodies directed only to the nascent chains; instead the double antibody technique was required for efficient precipitation. This behavior raises a problem since it is characteristic of a monovalent antigen, while the binding data quoted below show that the polysome is multivalent. A polysome is composed of about six ribosomes (Williamson and Askonas, 1967) each carrying one nascent L chain. Some of these may be too short to anchor the antibody molecule. Yet, the evidence indicates that 80% of the polysomes bind anti-C antibodies, and it seems reasonable to assume that each of these polysomes would also bind anti-V antibodies (either in the same nascent chain, or on another chain in the same polysome). Thus, in terms of antigenic constitution, most of the polysomes would be at least bivalent. Palmiter *et al.* (1972) found that several anti-ovalbumin antibodies were bound to one polysome of chicken oviduct. Nevertheless, in precipitation reactions the polysomes behaved as a monovalent antigen. The items discussed below may shed light on this problem. (1) Because of the large size of the polysome the distance between the antigenic determinants (*i.e.*, nascent chains) is much larger than it is on a usual protein antigen. This may cause difficulties in the formation by antibodies of the three-dimensional lattice required for precipitation. (2) Immunochemical studies on staphylococcal nuclease suggest that incomplete nascent chains, although capable of binding antibodies, have limited capacity in causing precipitation. This enzyme is composed of 149 amino acid residues. It has been shown that a large fragment (residues 1–126), which lacks conformation of the native enzyme but possesses 85% of its linear structure, binds to antinuclease antibodies (Omenn *et al.*, 1970b) but does not precipitate (Taniuchi and Anfinsen, 1969). (3) The effective valency of an antigen is the maximal number of antibody molecules which it can bind simultaneously. As expected this number increases with increasing molecular weight of the antigen. The valency of IgG was found to be 7; for free L chain it would be about 4 (Singer, 1965) but as a nascent chain it should have a lower value. Thus, the attachment of IgG (anti-L-chain) to a polysome increased its valency with respect to the anti-antibody. The theory of antigen–antibody reaction predicts that the probability of aggregate formation and the precipitation zone would increase with an increase in the valency of the antigen (Singer, 1965).

The experiments with anti-C and anti-V antibodies discussed previously are also relevant for specific problems involved in Ig biosynthesis. The distribution of genetic markers in the Ig chains raised the possibility that separate structural genes code for the variable and constant regions of the L chain (Cohn, 1968; Koshland *et al.*, 1969). Recently, Schubert and Cohn (1970) presented evidence suggesting that the variable and constant regions might be linked at the peptide level. This mechanism requires the existence of separate mRNA species coding for these regions. The finding that the molecular weight profile of products directed by mRNAs extracted from polysomes precipitated with anti-L-chain, anti-V, anti-C, anti-V_1C_2, and anti-C_1V_2 antibodies were indistinguishable from each other (Figure 6) shows that at least in MOPC-321 myeloma one mRNA molecule codes for the complete L chain. The argument is as follows. Mature MOPC-321 L chain has a molecular weight of 24,020 (calculated from sequence data, McKean *et al.*, 1973a), while the cell-free products were observed to have molecular weights in the range of 17,200–28,700. Let us assume that one of the smaller cell-free products was a variable region plus an extra piece (to account for an increase in molecular weight from 12,000 to 17,200). If this were the case, then this band should have been missing from products directed by mRNA prepared with anti-C, but it should have been the major product directed by mRNA from the residual polysomes precipitated with anti-V (the anti-C_1V_2 preparation). This is clearly contrary to the data presented in Figure 6. It should be noted, however, that our findings do not contradict the two genes one polypeptide hypothesis. The presumed variable and constant region genes could be linked at the DNA level, alternatively, the two genes could be transcribed separately but their mRNA products were linked before reaching the polysomes.

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