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# Biologically and Chemically Pure mRNA Coding for a Mouse Immunoglobulin L-Chain Prepared with the Aid of Antibodies and Immobilized Oligothymidine

**(mouse myeloma/mRNA molecular weight/L-chain precursor proteins/ partial amino-acid sequence of precursor/sequence of cell-free product)**

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**ABSTRACT** **The mRNA coding for a mouse immunoglobulin L-chain was prepared from MOPC-321 myeloma polysomes specifically precipitated with antibodies directed against L-chains, followed by chemical purification on oligo(dT)-cellulose. Biological purity (capacity to program the synthesis only of L-chain) was calculated to be $\geq 95\%$. 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. A similar degree of purity was calculated from the extent of precipitation of myeloma and nonmyeloma polysomes with anti-L-chain and non-L-chain antibodies. Chemical purity (95%) was determined from the amount of rRNA in the mRNA preparation by scanning of appropriate gels. In a cell-free system, the purified mRNA directed the synthesis of two precursors heavier than L-chain by about 1300 and 4700 daltons. Cell-free products labeled with 10 [$^{14}$C]aminoacids yielded 27 out of 28 expected L-chain tryptic peptides and four additional peptides. Most probably the latter were derived from extra pieces in the precursors, and the apparent loss of one peptide was due to modifications at the N-terminus. The main fraction of L-chain mRNA was composed of two species of about 420,000 and 450,000 daltons. These molecules are much larger than that required to code for a mature L-chain (calculated about 250,000). The additional nucleotide mass can be accounted for in part for the coding of the extra piece (about 50,000) and in part for the polyadenylate moiety.**

Recently, several laboratories have reported the isolation of immunoglobulin L-chain mRNA from various mouse myelomas (1–6). The procedures were based on three principles: (*1*) The use of microsomes, presumably enriched with polysomes engaged in L-chain synthesis, as the source for RNA extraction (1–3, 5, 6). (*2*) Isolation of the 12–14S RNA fraction by sucrose-gradient centrifugation (1–3, 5, 6). (*3*) Selection for poly(A)-rich RNA (2, 4, 6). The above procedures might have led only to partial purification because the microsomes still synthesize proteins other than L-chain (2, 5, 7). Furthermore, as shown here, the 12–14S RNA fraction extracted from myeloma polysomes (although not microsomes) is heavily contaminated with non-L-chain mRNAs, and the biological activity of the poly(A)-enriched RNA is indistinguishable from that of the crude RNA preparation.

Although it has been repeatedly shown in several systems that antibodies can bind to incomplete nascent chains on polysomes (for a recent review see ref. 8), previous attempts to purify mRNAs with antibodies have met with limited success (9). Recently, Palacios *et al.* (10) reported on the use of antibodies for preparation of an RNA fraction that is highly enriched with ovalbumin mRNA.

It is shown in the present study that, with the aid of antibodies directed against immunoglobulin L-chain, it is possible to achieve specific precipitation of polysomes that yield biologically pure mRNA ($\geq 95\%$). Criteria more rigorous than have been used previously, have been used to define the purity of the mRNA. Products programmed by the mRNA were labeled with 10 [$^{14}$C]aminoacids so that all L-chain peptides and even small amounts of non-L-chain contaminants could be identified (in contrast to previous works where only one labeled amino acid was used). Partial amino-acid sequence was also performed on one of the cell-free products.

## MATERIALS AND METHODS

The MOPC-321 myeloma (a kappa L-chain producer) was maintained as a solid tumor in female Balb/c mice (11). L-Chains were prepared from mouse urine (11), and tRNA was prepared from Krebs II ascites cells (12). [$^{3}$H]Leucine (35 Ci/mmol) and $^{14}$C-labeled amino acids (90–320 Ci/mol) were purchased from New England Nuclear Corp.

*Preparation of mRNA from Total Polysome Population.* MOPC-321 tumors of 17–20 days 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 ice-cold solution A [50 mM Tris·HCl (pH 7.4), 25 mM KCl, 5 mM $MgAc_2$, 7 mM 2-mercaptoethanol] and homogenized first in a Waring Blendor and then in a Dounce homogenizer. Nuclei and debris were removed by centrifugation at 20,000 × *g* for 20 min; aliquots of the supernatant (15 ml) were layered over a cushion of 1.5 M sucrose in solution A (11 ml) and spun (40,000 rpm for 10 hr) at 2° in a Spinco 60Ti rotor, and the polysomal pellet was suspended in 0.25 M sucrose in solution A (yield, 130–150 $A_{260}$ units of polysomes per g of tumor). RNA was extracted by shaking for 10 min at room temperature (23°) equal volumes of water-saturated phenol and a solution containing: 65 $A_{260}$ units/ml of polysomes, 1.5% sodium dodecyl sulfate, 100 mM Tris·HCl (pH 9), 100 mM sucrose, 10 mM KCl, 2 mM magnesium acetate, and 3 mM mercaptoethanol. The aqueous phase was separated, and the phenol phase was extracted once with 1 volume of 0.5% sodium dodecylsulfate–10 mM Tris·HCl (pH 9). After centrifugation, the aqueous phases were combined and re-extracted three times with phenol, and the RNA was precipitated by addition of 0.1 volume each of 2.0 M NaCl and of 2.0 M sodium acetate (pH 5.5), and 2.5 volumes

Abbreviations: I.P RNA, RNA extracted from immune precipitated polysomes; I.P.T RNA, I.P RNA purified on oligo(dT)-cellulose; o-T RNA, RNA extracted from total polysome population and purified on oligo(dT)-cellulose.

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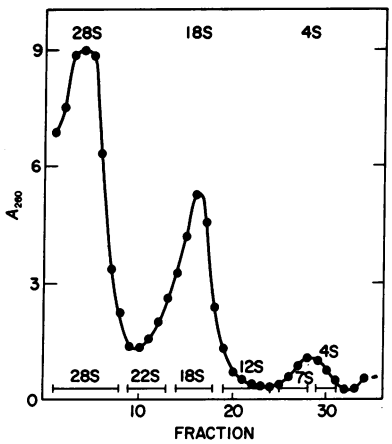


FIG. 1. Sucrose-gradient sedimentation of total polysomal extract. *Horizontal bars* indicate fractions pooled.

of ethanol at −20°. The pelleted RNA was washed once in the above medium (NaCl–sodium acetate–ethanol), dissolved in solution B [0.4% sodium dodecyl sulfate, 100 mM NaCl, 10 mM Tris·HCl (pH 7.4), and 1 mM EDTA], layered over a 15–30% sucrose gradient made in solution B, and centrifuged (27,000 rpm/14 hr) at 22° in a Spinco SW27 rotor. A typical ribosomal RNA profile was obtained. Regions corresponding to about 4 S, 7 S, 12 S, 18 S, 22 S, and 28 S were pooled (Fig 1), and the RNA was precipitated, washed repeatedly to remove traces of sodium dodecyl sulfate, dissolved in water, and stored at −70°. The 12S and 22S RNA fractions were further purified by passage on oligo(dT)–cellulose (19).

*Preparation of mRNA from Immune-Precipitated Polysomes.* The double-antibody precipitation technique used to precipitate polysomes carrying nascent immunoglobulin L-chains (manuscript in preparation) was, briefly: the total polysome population from the 1.5 M sucrose cushion (see above) was reacted first with goat anti-MOPC-321 L-chain and then with rabbit anti-goat immunoglobulin. The precipitate that contained antigen–antibody complexes as well as polysomes was treated with sodium dodecyl sulfate–phenol, and the extracted RNA was fractionated over a 15–30% sucrose gradient and purified on oligo(dT)–cellulose as described above. Polysomes from 150 g of tumor could be easily processed in one batch.

*Cell-Free Translation of mRNA.* The Krebs II ascites system (12, 13) was used with some modifications. One reaction mixture (62 μl) contained: 0.13 $A_{260}$ units of the 130,000 × g ribosome-free supernatant, 0.11 $A_{260}$ units of ribosomes, 0.15 $A_{260}$ units of tRNA, 21 mM Tris·HCl (pH 7.4), 3.7 mM magnesium acetate, 30 mM KCl, 24 mM $NH_4Cl$, 0.8 mM ATP, 0.08 mM GTP, 0.4 mM CTP, 8 mM creatine phosphate, 5 mM 2-mercaptoethanol, 13 μg/ml of creatine phosphokinase, 5 μM [$^3$H]Leu, or 7 μM [$^{14}$C]Leu, or a mixture of 10 [$^{14}$C] aminoacids (Ala, Arg, Gly, Ileu, Leu, Lys, Pro, Ser, Thr, and Val, each at 2.7 μM) complemented with 19 or 10 unlabeled aminoacids (each at 30 μM), as required. Amounts of mRNA and incubation time at 36° are indicated. $Cl_3CCOOH$-precipitable material was measured on a Millipore filter (12).

[$^{14}$C]*Leu-Labeled Marker.* MOPC-321 tumor fragments in Eagle no. 2 medium containing 10 μCi/ml of [$^{14}$C]Leu (300

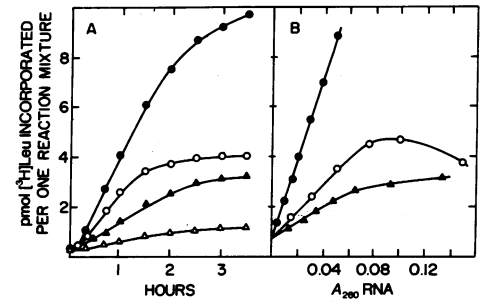


FIG. 2. Translation of mRNAs extracted from total polysomes in the Krebs II cell-free system as a function of time (*A*) and concentration (*B*). In *A*, a reaction mixture (62 μl) contained 0.037, 0.029, or 0.032 $A_{260}$ units of 7S, 12S, or 22S mRNA, respectively. In *B*, reaction mixtures were kept for 75 min. Symbols for mRNA added: ●, 12 S; ○, 7 S; ▲, 22 S; △, none.

Ci/mol) were kept at 37° for 4 hr. The L-chain protein was purified from the tumor homogenate by chromatography on DEAE-A25 (11) and Sephadex G-100 columns. This marker (780,000 cpm/mg) was shown to be an authentic L-chain by polyacrylamide-gel electrophoresis, by fingerprint analysis of a tryptic digest (Found: 11 radioactive spots, all of which matched with ninhydrin-stained peptides derived from unlabeled L-chain, see Fig. 5. Calculated: 9 spots, from ref. 14), and by amino-acid sequence analysis (45 steps, radioactivity detected in steps 4, 11, and 15, as expected from chemical sequence, ref. 14).

## RESULTS

### mRNAs from total polysome population

The RNA profile of polysomal extract is given in Fig. 1. In a cell-free system, mRNA activity was detected in fractions

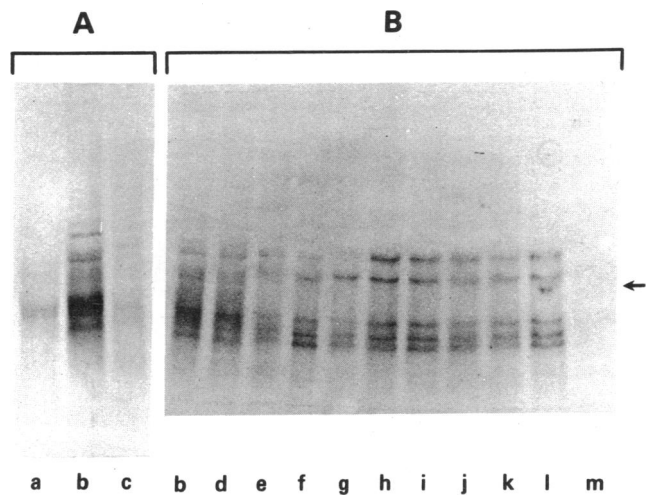


FIG. 3. Autoradiograms of sodium dodecyl sulfate–polyacrylamide gels of cell-free products. Total reaction mixtures labeled with 10 [$^{14}$C]aminoacids were reduced and analyzed essentially as described (20). The products were programmed by the following mRNAs: in slab *A* (15% acrylamide, 16 V/cm, 2 hr): *a*, 7 S; *b*, 12 S; *c*, 22 S. In slab *B* (13% acrylamide, 16 V/cm, 1.5 hr): *b*, 12 S; *d*, 12S o-T; *e*, 22S o-T; *f*, 12S I.P; *g*, 22S I.P; *h*, 12S I.P.T; *i*, 18S I.P.T; *j*, 22S I.P.T; *k*, 28S I.P.T; *l*, same as *h*, plus a marker of [$^{14}$C]Leu-labeled L-chain; *m*, none. *Arrow* indicates the position of the marker. Molecular weight standards were: ovalbumin, glyceraldehyde phosphate dehydrogenase, MOPC-321 L-chain, myoglobin, and hemoglobulin.

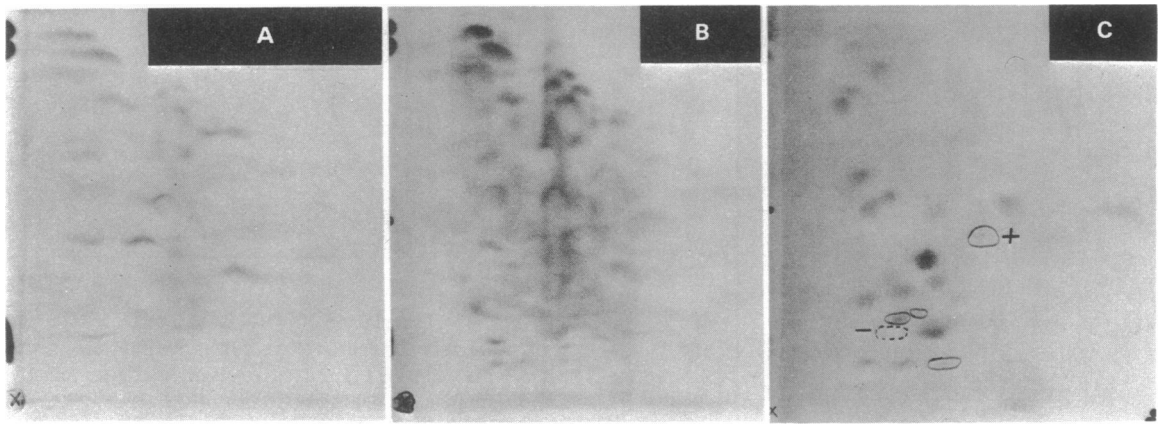


FIG. 4. Autoradiograms of fingerprints of 10 [$^{14}$C]aminoacid-labeled cell-free products. Five reaction mixtures kept at 36° for 4 hr and supplemented with 2 mg of L-chain were aminoethylated (21), digested with TPCK-trypsin (E:S ratio of 1:65), loaded on Whatman no. 3 paper, and analyzed by chromatography (butanol–acetic acid–$H_2O$, 17 hr) and then by electrophoresis at pH 3.5 (3000 V/50 min). After exposure to x-ray film, the paper was stained with an arginine-specific reagent and then with acid ninhydrin (22). Spots in autoradiogram and stained paper were matched. mRNAs present in the reaction mixtures were: *A*, 7 S; *B*, 12 S; *C*, 12S I.P.T. In *C*, 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 Fig. 5. Peptides encircled with a *continuous line* are not detected in Fig. 5. The region encircled with a *dashed line* is empty here but occupied by a peptide that contains both leucine and arginine in Fig. 5.

corresponding to 7S, 12S, and 22S regions of the gradient (Fig. 2) but essentially none was found in the 4S, 18S, and 28S fractions. Polyacrylamide gel of the cell-free products showed that the 7S mRNA programmed the synthesis of one protein of about 20,000 daltons. The 12S and 22S mRNAs yielded a few proteins ranging in size from about 30,000–17,000 daltons (Fig. 3*A*). Tryptic digest of the 7S product did not contain L-chain peptides (Figs. 4*A* and 5). The 12S products yielded many peptides; some were identical with peptides derived from the 7S products, others were of L-chain origin (identified in regions where the 7S autoradiogram was relatively free of peptides, compare Figs. 4*A*, *B*, *C*, and 5). After purification on oligo(dT)–cellulose the specific activity of the 12S and 22S mRNAs was increased (Table 1). However, their biological activities were not altered since the same protein bands were programmed before and after purification (see Fig. 3*A* and *B*). In addition, the fingerprint of products stimulated by 12S o-T mRNA [extracted from total polysome population and purified in oligo(dT)–cellulose; data not shown] was identical with that derived from crude 12S mRNA (Fig. 4*B*).

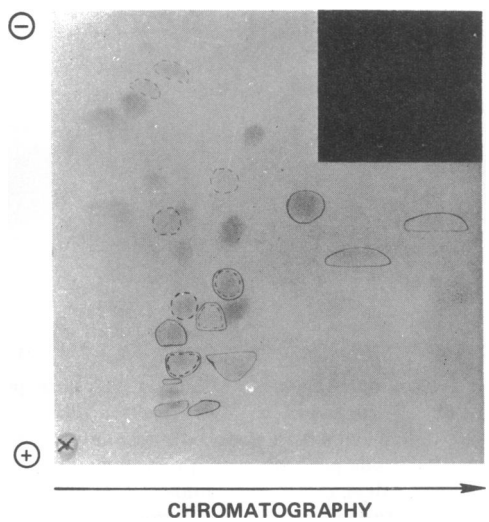


FIG. 5. Fingerprint of unlabeled MOPC-321 L-chain. Fingerprint of the tryptic digest was prepared and stained as described in Fig. 4. Arginine-containing peptides are encircled with *dashed lines*. Leucine-containing peptides are encircled with *continuous lines*. These were determined from autoradiogram of fingerprint of [$^{14}$C]Leu-labeled L-chain marker.

**mRNA from immune-precipitated polysomes**

From the total MOPC-321 polysome population the fractions precipitated were: 17.3% with goat anti-L-chain, 1.9% with normal goat immunoglobulin (in both cases the same amount of antigen–antibody precipitate was formed by addition of rabbit anti-goat immunoglobulin), and 0.7% without any antibody added. Furthermore, when Krebs II ascites polysomes were similarly treated with the two antigen–antibody systems, equal and low amounts of polysomes were precipitated (2.0 and 2.1%). The specificity ratio for precipitation would increase from 14 [(17.3–0.7)/(1.9–0.7)] to 20 if we consider that about 30% of the material that coprecipitated with unrelated antibodies may not have carried functional mRNA (single ribosomes and oligosomes with defective mRNA).

The sedimentation profile of RNA extracted from immune-precipitated polysomes was similar to that of RNA obtained from regular polysomes. In a cell-free system, considerable mRNA activity was detected in the 12S and 22S fractions but none in the 7S fraction. A very low activity could be detected in the 18S and 28S fractions. The crude 12S–28S I.P mRNA preparations were purified on oligo(dT)–cellulose (I.P.T mRNA; yields and specific activities of the I.P.T mRNAs obtained are given in Table 1). Polyacrylamide gels showed that identical products were programmed by I.P and I.P.T mRNAs (Fig. 3*B*). In all cases, five protein bands were observed, and their mobilities (corrected for protein load on the gel by including [$^{14}$C]Leu-labeled marker in the cell-free sample) corresponded to molecular weights of 28,700, 25,300,

19,700, 18,200, and 17,200. On long exposure, a faint band comigrating with authentic L-chain (molecular weight, 24,020, calculated from sequence data, ref. 14) was detected. Tryptic digests of the products of 12S and 22S I.P mRNAs and of all four I.P.T mRNAs yielded identical fingerprints, one of which is shown in Fig. 4C. By matching the autoradiograms with the ninhydrin-stained fingerprint of authentic L-chain, it was found that only one L-chain peptide was missing, and that four, and perhaps five, additional peptides were present in the cell-free digests.

**Polyacrylamide analyses of mRNAs**

In a nondissociating medium, the I.P.T mRNAs were very heterogeneous (Fig. 6A). In addition to various amounts of 18S and 28S ribosomal RNA, the preparations contained several bands ranging in size from 15.5–34.5 S. Phenol can cause aggregation of RNA (15), and since it was used for extracting the myeloma polysomes, gel electrophoresis was also done in a dissociating medium (3.6% acrylamide in 98% formamide, ref. 17). The results (Fig. 6B) showed that the non-rRNA bands with S values larger than 18 have been reduced to two closely adjacent bands with mobilities corresponding to molecular weights of about 420,000 and 450,000 (about 15.5 S). To ascertain the validity of these measurements, the 22S I.P.T mRNA was analyzed in formamide gels at three different acrylamide concentrations (3.2%, 3.6%, and 4.0%) for 5 hr, and at 3.6% acrylamide for 12 hr; one sample of the mRNA was dissolved in 100% dimethylsulfoxide (a strong disaggregating agent, ref. 18) before it was put on the gel. The mobilities changed as expected, but the same molecular weights were calculated. Also, at the 12-hr run, the two closely adjacent bands were clearly separated.

TABLE 1. *Summary of oligo(dT)–cellulose purification of MOPC-321 mRNAs**

| mRNA loaded | Poly(A)-enriched mRNA fraction: Yield (%) | S.A.† (pmol of Leu per 0.01 $A_{260}$/1 hr) Observed | S.A.† (pmol of Leu per 0.01 $A_{260}$/1 hr) Corrected | Relative specific activity Observed | Relative specific activity Corrected | Specific activity ratio‡ |
|---|---|---|---|---|---|---|
| 12S | 14.2 | 3.9 | | 1.4 | | 3.7 |
| 22S | 5.6 | 1.2 | | 0.4 | | 4.7 |
| 12S I.P | 10.0 | 2.8 | 3.0 | *1.0* | *1.0* | 3.7 |
| 18S I.P | 2.7 | 2.3 | 3.5 | 0.8 | 1.2 | ~10§ |
| 22S I.P | 4.1 | 1.5 | 3.3 | 0.5 | 1.1 | 5.0 |
| 28S I.P | 0.9 | 1.1 | 4.2 | 0.4 | 1.4 | ~5§ |

* RNA fractions obtained from sucrose gradients were purified on oligo(dT)–cellulose columns essentially as described (19). The data refer to the poly(A)-enriched material that was retained on the column at high salt (0.5 M KCl) and was eluted at low salt [5 mM Tris·HCl (pH 7.4)]. These eluates are designated in the *text* as o-T or I.P.T mRNAs, depending on the source of RNA loaded.

† Specific activity calculated from kinetic slopes under conditions where the reaction was first order with respect to time and concentration (see Fig. 2). For calculation of observed specific activity, the total $A_{260}$ value of the RNA was used; for corrected specific activity, only the fraction of RNA (peaks and shoulder) with mobility of 15.5–9.5 S in formamide gel was used (see *text*).

‡ Observed ratio of specific activity after/before passage on oligo(dT)–cellulose.

§ Before samples were put on the column activity was very low and kinetic slopes could not be established with certainty.

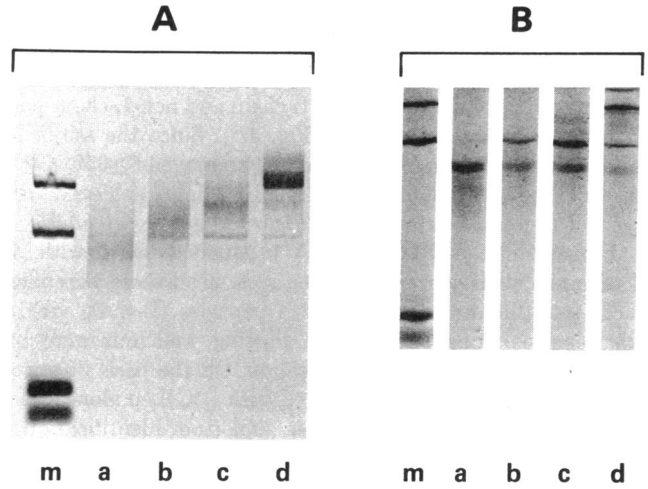


FIG. 6. Gel electrophoresis of L-chain mRNAs. Samples were analyzed on gels composed of: *A*, 1.7% acrylamide–0.5% agarose in aqueous medium, 250 V/1.5 hr (16); *B*, 3.6% acrylamide in 98% formamide, 100 V/5 hr (17), and developed with "stain all" (16). RNA samples are: *m*, 28, 18, 5, and 4 S from MOPC-321 ribosomal RNA; *a*, 12S I.P.T; *b*, 18S I.P.T; *c*, 22S I.P.T; *d*, 28S I.P.T.

The stained formamide gels (3.6% acrylamide, 5 hr) were scanned in a Gilford spectrophotometer at 570 nm. The total area scanned accounted for three components: rRNA contamination, the two barely separable major bands (about 15.5 S), and a shoulder of RNA that extended from the 15.5S region down to the 9.5S region. In all cases, the area under the shoulder (15.5–9.5 S) was about equal to that occupied by the two adjacent peaks (15.5 S). Fractions of non-rRNA (i.e., the 15.5S plus the 15.5–9.5S shoulder) in the 12S, 18S, 22S, and 28S I.P.T mRNA preparations were, respectively, 95, 66, 45, and 26%.

## DISCUSSION

By use of antibodies for the preparation of biologically pure mRNA from cells that contain a multitude of different mRNA species, two problems are encountered. (*1*) Achievement of specific precipitation only of polysomes carrying nascent chains of the protein in question. (*2*) Definition of the reaction conditions under which the mRNA attached to the polysomes would retain its full biological activity. In the present investigation, these requirements were met, as evident from the data given in Figs. 3–5 and Table 1 and from the complete lack of non-L-chain mRNA activity in the 7S RNA fraction of immune-precipitated polysomes. The biological purity of the L-chain mRNA is estimated to be $\geq 95\%$. This is based on polysome precipitation (see *Results*) and autoradiogram data. Peptides derived from the product of the non-L-chain 7S mRNA that contaminate the fingerprint derived from 12S mRNA can be used as markers for purity. On prolonged exposure, some (but not all) of the contaminating (non-L-chain) peptides were observed at low intensities in fingerprints programmed by the 12S I.P.T mRNA. This occurred only when the intensities of L-chain peptides of the 12S I.P.T mRNA

were 25-fold stronger than the autoradiograms of the corresponding L-chain peptides directed by the nonimmune-precipitated 12S mRNA. The latter RNA fraction programmed the synthesis of both L-chain and non-L-chain peptides in comparable amounts (Fig. 4*B*). Since the major 7S mRNA(s) contaminant was effectively removed ($\geq 95\%$), it is conceivable that similar purification was also achieved with respect to minor contaminants that could not be detected.

Fingerprints of I.P.T mRNA products labeled with 10 [$^{14}$C]aminoacids contained 31 peptides. 27 of these were identical to authentic L-chain-derived peptides (L-chain yielded 28 peptides), one peptide was missing, and four were not present in the authentic hydrolyzate. On the basis of fingerprints of cell-free products labeled with [$^{14}$C]Leu alone and of [$^{14}$C]Leu-labeled L-chain marker, and from identification of arginine-containing peptides, it was shown that the missing peptide corresponded to a L-chain peptide containing leucine and arginine (details to be published). The amino-acid sequence of MOPC-321 L-chain shows that tryptic digestion would yield an N-terminal peptide with Leu and Arg residues (14). The data quoted below suggest that the apparent loss of one peptide may be due to the fact that I.P.T mRNAs direct the synthesis of L-chain precursors (see refs. 2, 4, and 6) in which the N-terminal end of authentic (mature) L-chain is modified by the attachment of an extra run of peptide chain.

Two proteins of the cell-free products were heavier than authentic L-chain by about 4700 and 1300 daltons. The possibility that these are not L-chain precursors but represent unrelated proteins is very low indeed because these bands constituted about 50% of the total cell-free products (Fig. 3*B*) and the fingerprint of the total reaction mixture was composed almost entirely of L-chain peptides (Fig. 4*C*). Nevertheless, products of 12S I.P.T mRNA labeled with [$^{3}$H]Leu were separated on a cylindrical polyacrylamide gel. A radioactive band with mobility slower than that of L-chain was eluted (unfortunately, it was not possible to decide which of the two heavier bands was eluted because of technical problems in the parallel gel that contained protein markers). In a preliminary experiment, the eluate (165,000 cpm) was sequenced (60 steps) in a Beckman Automatic Sequencer (unpublished data, I. Schechter, R. Guyer, and W. Terry). Radioactivity was detected at steps 6, 7, 8, 11, 12, 13, 24, 31, and 35 (MPOC-321 L-chain contains Leu residues at positions 4, 11, 15, 50, etc., ref. 14). Thus, it may tentatively be concluded that one of the precursors contained at the N-terminal end an extra piece composed of 20 amino-acid residues, 30% of which are leucines. Since in this experiment it was not possible to establish which of the two heavy precursors was analyzed, the possibility of elongation at the C-terminus is still open to investigation.

Polyacrylamide gels (in aqueous and in dissociating media, Fig. 6) showed that the 12S I.P.T mRNA is composed of two closely adjacent bands of 15.5 S (43%), of a smear of RNA species ranging in size from 15.5–9.5 S (52%), and of 18S rRNA (5%). Because of the strong biological activity of this preparation (Figs. 3 and 4, and Table 1) and the fact that about equal amounts of the cell-free products were heavier or lighter than L-chains (Fig. 3*B*), it is suggested that the 15.5S mRNAs code for the precursors, and the 15.5–9.5S mRNAs code for the smaller fractions (this can be tested by extraction of the mRNAs from the gel and subsequent analysis of the products they program). Partial degradation of 15.5S mRNA down to 9.5S species is probably not due to treatment with antibodies, since polysomes that underwent two cycles of the antibody purification procedure yielded mRNA that programmed identical protein pattern. The mRNA degradation presumably occurred at an earlier stage, during polysome isolation, or in the intact cell.

The L-chain mRNA has a high tendency for aggregation, probably due to contact with phenol. In the nonaggregated state (12S I.P.T mRNA) and in the aggregated state (18S, 22S, and 28S I.P.T mRNAs) the mRNAs had identical biological activity (Figs. 3*B* and 4*C*), but they differed in their specific activities. However, by correcting for the extent of contamination of each fraction with rRNA, it turned out that all mRNAs had similar activity (see Table 1).

It appears that the L-chain mRNA described here would serve as a reliable tool for the quantification of immunoglobulin gene-dosage, and for the study of control of immunoglobulin production at the levels of transcription and translation. However, nucleotide-sequence analysis will be required to provide ultimate proof for chemical purity. In addition, since in this study unique properties of the antigen or antibody were not required, it seems that the antibody approach might serve as a general solution for the isolation of biologically pure mRNAs from fully functional eukaryotic and prokaryotic cells.

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