AGGREGATES OF PARTIALLY PURIFIED mRNA CODING FOR IMMUNOGLOBULIN LIGHT-CHAIN

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SUMMARY. The 12S and 22S RNA fractions were isolated from phenol extract of the total polysome population of MOPC-321 mouse myeloma (a light-chain producer). Both RNA fractions exhibited mRNA activity when tested in the Krebs II ascites cell-free system. Fingerprint analyses of tryptic digests of the cell-free products showed that whereas the 12S RNA fraction contained L-chain mRNA together with a large amount of non-L-chain mRNAs, the 22S RNA fraction showed almost exclusively L-chain mRNA activity. The observed purification that was achieved by sucrose gradient centrifugation might be attributed to the tendency of L-chain to form aggregates upon exposure to phenol.

A pure mRNA molecule coding for immunoglobulin would be a useful tool for studying the origin of antibody diversity and the regulation of Ig-gene expression. Several laboratories reported the isolation of preparations containing Ig L-chain mRNA from various mice myelomas (1-7). In these studies the search was concentrated on the 11-14S RNA fraction that was isolated from the phenol extract of total microsome population of the tumor. This fraction was chosen because the calculated size of RNA molecules coding for the L-chain protein [assuming three nucleotides per amino acid plus poly(A) segment] should fall within this range size.

Recently rigorous criteria were applied to analyse the proteins programmed by myeloma mRNAs. These included: analysis of the total cell-free reaction mixture (in contrast to products selected from the reaction by specific precipitation with antibody), two dimensional fingerprint analysis of the products (one dimension was used before), products were labeled with ten [^{14}C]amino acids so that all peptides could be detected (one labeled amino acid was used before). This study showed that the 11-14S RNA fraction which contained L-chain mRNA was heavily contaminated with non-L-chain mRNA activities (8). In the present report it is shown that the 22S RNA fraction isolated from RNA of total polysome population contains a relatively pure L-chain mRNA.

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MATERIALS AND METHODS. The MOPC-321 myeloma (gift of Dr. M. Potter) was kept as a solid tumor in female Balb/c mice. The preparation of total polysome population from tumor tissue, RNA from the phenol extract of polysomes, and the modified Krebs II ascites cell-free system used to assay and translate the mRNA preparations were described (8). The preparation of pure L-chain mRNA from polysomes specifically precipitated with antibodies to L-chain was described elsewhere (9) and experiments showing its biological purity ($\geq$95%) are given in ref. 8.

The RNA extract of total polysome population was fractionated on a 15-30% sucrose gradient made in 100mM NaCl-10mM Tris·HCl (pH 7.4)-1mM EDTA, and centrifugation at 22° in a Spinco SW27 rotor. The 12S and 22S RNA fractions from several runs were pooled and purified over oligo(dT)-cellulose columns essentially as described (10). The poly(A)-rich RNA fractions obtained refer to the RNA that was retained on the column at 0.5M KCl-0.01M Tris·HCl (pH 7.4) and eluted at 5mM Tris·HCl (pH 7.4).

RESULTS. The RNA profile of polysomal extract is given in Fig. 1. In the Krebs II cell-free system, mRNA activity was detected in fractions corresponding

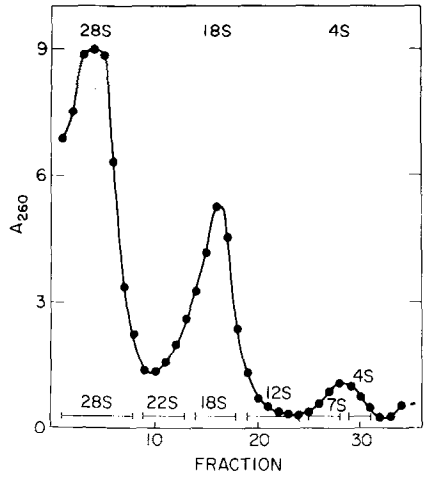

Fig. 1. Sucrose gradient centrifugation (27,000 rpm/14 hr) of total polysomal extract. Horizontal bars indicate fractions collected.

to the 7S, 12S and 22S regions of the gradient, but essentially none was found in the 4S, 18S and 28S fractions. In a previous report it was shown that the mRNA activity in the 7S RNA fraction corresponded to a protein (not yet identified) other than L-chain (8). The mRNA activity of the 22S fraction was some-

Table I. mRNA activity in RNA fractions

RNA fraction	12S			22S			18S*
	1x cent.	2x cent.	poly(A) -rich	1x cent.	2x cent.	poly(A) -rich	2x cent.
pmoles Leu incorporated†	1.22	3.28	8.44	0.54	1.02	3.12	0.11

The RNA obtained from the original sucrose gradient (1x cent.), from repeated centrifugation (2x cent.), and the poly(A)-rich RNA were assayed in the Krebs II cell-free system (0.015 A_{260} RNA per reaction). Incorporation of [^{3}H]leucine into TCA precipitable material was measured after 1.5 hr at 36° (8).

* 18S RNA isolated from either the 12S or 22S RNA fractions (see Fig. 2).

† Net incorporation obtained by substracting 0.65 pmoles for the minus RNA control.

what low (Table I) and therefore it was rerun on a sucrose gradient. As seen from Fig. 2 the original 22S RNA was resolved into two peaks of about 22S and 18S that were collected separately. The original 12S fraction was also rerun and it resolved into 12S and 18S peaks. This treatment increased the mRNA activity in the 12S and 22S fractions, correspondingly, the 18S RNA resolved from either the 12S or 22S fractions had negligible activity (Table I).

The autoradiogram of fingerprints of cell-free products directed by the 12S and 22S RNA fractions were different (Fig. 3). The 12S product yielded many peptides; a few were of L-chain origin (identified by overlap with ninhydrin-stained fingerprint of cold L-chain), many others were non-L-chain peptides and some of these were identical to peptides derived from products directed by the 7S non-L-chain mRNA (see ref. 8). In contrast, in the fingerprint of the 22S product the vast majority of non-L-chain peptides were undetectable, a few non-L-chain peptides were very faint. By matching the 22S autoradiograms (Fig. 3C,D) with the ninhydrin-stained fingerprint of cold L-chain (Fig. 3F) it was found that only one L-chain peptide was missing and that about 4 additional peptides were present in the cell-free digest. The same missing and additional peptides were recorded in the fingerprint of products programmed by a biologically pure L-chain mRNA (Fig. 3E and ref. 8).

On polyacrylamide gels the 22S RNA yielded five protein bands ranging in size from 28,700 to 17,200 daltons (Fig. 4C,D). This pattern was indistinguishable from that obtained with products directed by the pure L-chain mRNA (Fig. 4E). The 12S RNA yielded a somewhat different pattern that contained a few additional bands (Fig. 4A,B).

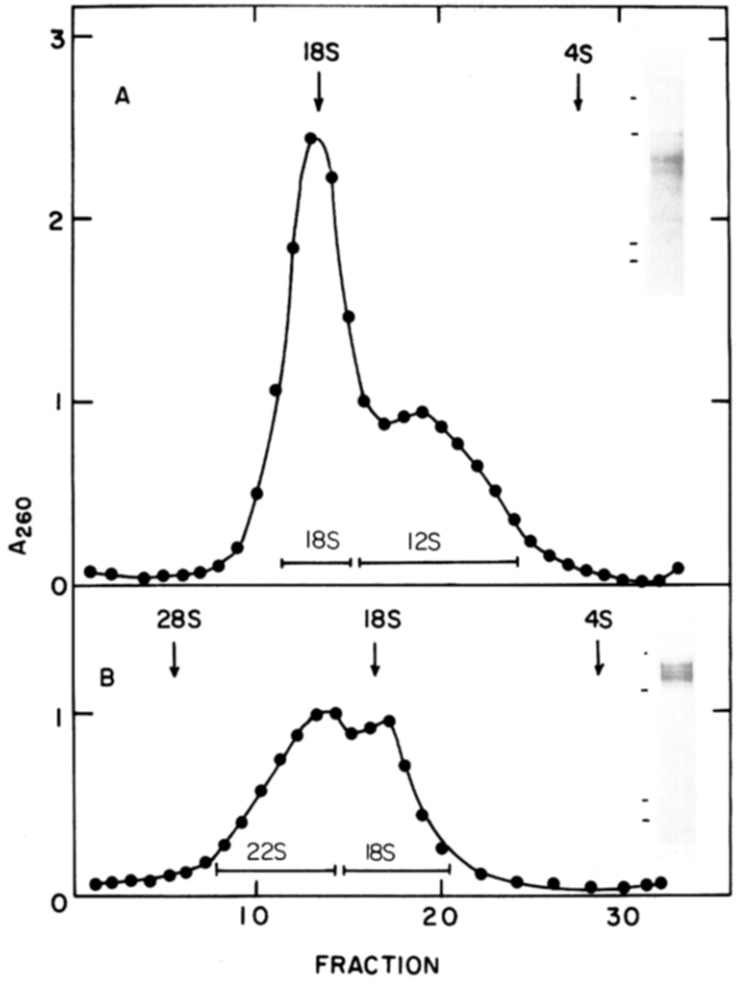

Fig. 2. Sucrose gradient centrifugation of the 12S (in A, 27,000 rpm/18 hr) and 22S (in B, 27,000 rpm/14 hr) RNA fractions indicated in Fig. 1. Horizontal bars indicate fractions collected. Vertical arrow indicate the positions of RNA markers. Inserts: gel electrophoresis (1.7% acrylamide - 0.5% agarose in aqueous medium, 250V/1.5 hr, ref. 13) of the 12S (in A) and 22S (in B) RNA fractions collected after the second run. Short bars on the side denote the position (from top to bottom) of 28, 18, 5 and 4S RNA markers.

The poly(A)-rich RNA obtained from the 12S and 22S RNA fractions had increased mRNA activity (Table I) but their biological purity was not changed. RNA before and after passage on oligo(dT)-cellulose yielded the same fingerprints (Fig. 3) and the same pattern of protein bands (Fig. 4).

DISCUSSION. A biologically pure L-chain mRNA was obtained in the form of large aggregates (up to 35.5S) that could be reduced to monomers (about 15.5S,

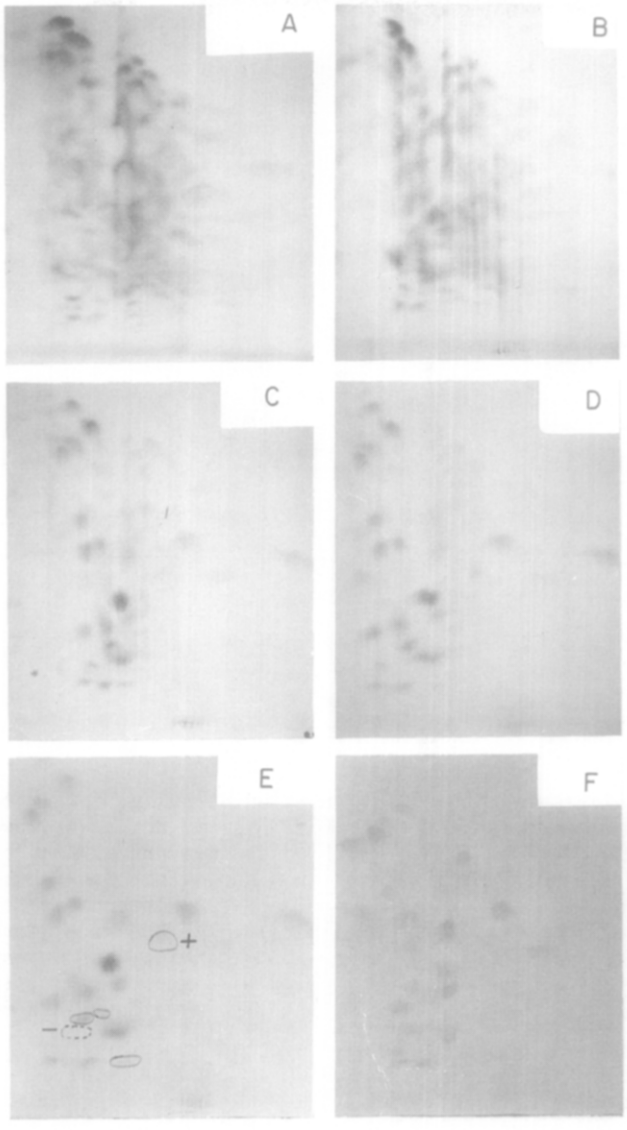

Fig. 3. Fingerprints of cell-free products (A to E) and of authentic MOPC-321 L-chain (F). Autoradiograms of products labeled with 10 [^{14}C]amino acids that were programmed by: A, 12S RNA from total polysome population; B, same as A after purification on oligo(dT)-cellulose; C, 22S RNA from total polysome population; D, same as C after purification on oligo(dT)-cellulose; E, 12S RNA extracted from polysomes specifically precipitated with antibodies to L-chain. Total cell-free reaction mixtures kept at 36° for 4 hr and supplemented with 2mg of MOPC-321 L-chain were aminoethylated (14), digested with TPCK-trypsin (E:S ratio of 1:65), loaded on a Whatman #3 paper, subjected to chromatography (n-butanol-acetic acid-water, 17 hr) followed by electrophoresis (pH 3.5, 3000V/50min), and exposure to X-ray film. Panel F, ninhydrin stained fingerprint of cold MOPC-321 L-chain marker. In pannel E, peptides encircled with continuous lines and the region encircled with a dashed line denote, respectively, the additional and missing peptides in the L-chain precursor programmed by the pure mRNA (8).

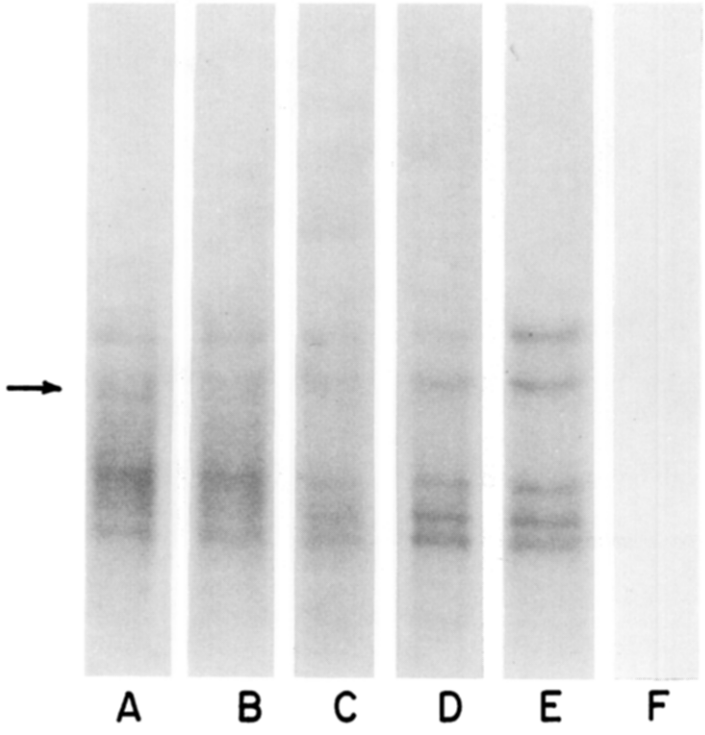

Fig. 4. Autoradiogram of SDS-13% polyacrylamide gel of cell-free products. Total reaction mixtures labeled with 10 [^{14}C]amino acids were reduced and analysed on gels as described (15). The products were programmed by: 12S RNA from total polysome population before (in A) and after (in B) oligo(dT)-cellulose purification; 22S RNA from total polysome population before (in C) and after (in D) oligo(dT)-cellulose purification; 12S RNA from immune precipitated polysomes (in E). In F, none, minus mRNA control. Arrow indicates the position of MOPC-321 L-chain marker applied with the cell-free sample. Molecular weight standards were: ovalbumin, L-chain, myoglobin and hemoglobin.

Fig. 5B,D) upon exposure to dissociating agents such as formamide (8) or dimethylsulfoxide (I. Schecther, unpublished data). The pure mRNA was prepared from the phenol extract of immune-precipitated polysomes, and the aggregation was probably due to contact with phenol which can cause aggregation of RNA (11). This property was preserved in the phenol extract of total polysome population since L-chain mRNA was detected in both the 12S and 22S RNA fractions. The non-L-chain mRNA species present in the 7S (8) and 12S fractions did not aggregate considerably (if at all) because they were hardly detected in the 22S RNA. Thus extensive purification of the L-chain mRNA was simply achieved by centrifugation of the phenol extract of total polysome population (Fig. 3). Similarly to pure L-chain mRNA, the 22S RNA on formamide gel yielded a prominant band migrating at

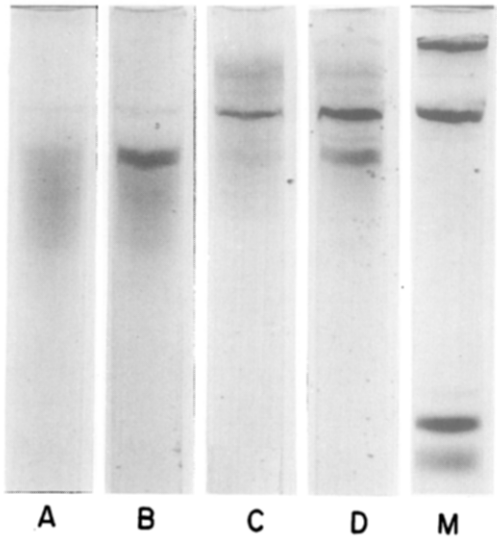

Fig. 5. Gel electrophoresis in a dissociating medium of poly(A)-rich RNA. Samples are: 12S (in A) and 22S (in C) mRNA from total polysome population; 12S (in B) and 22S (in D) pure mRNA from immune-precipitated polysomes. In M, 28, 18, 5 and 4S RNA markers. The gels, 3.6% acrylamide in 98% formamide, were run at 100V/5 hr (16).

15.5S (Fig. 5C). In the 12S RNA (Fig. 5A) this band could not be resolved, probably because of masking by the large amounts on non-L-chain mRNA species. However, it still remains to determine whether the 22S RNA (from total polysome population) contained non-L-chain mRNAs, each present in small amount, that could not be detected by fingerprint analysis. This can be done by using cDNA complementary to pure L-chain mRNA.

Upon repeated centrifugation of the 12S or 22S RNA fractions the L-chain mRNA remained in its original position and it was hardly shifted to the 18S RNA that was resolved (Fig. 2, Table I). This indicated that the mRNA aggregates were fairly stable; new ones were not formed and aggregates already present did not dissociate. It was shown before that by using antibodies over 95% of the non-L-chain mRNA species were removed from the 12S RNA fraction (Fig. 3A,B,E; ref. 8). In view of the present observation, it seems, in retrospective, that even higher biological purity was achieved in the L-chain mRNA that was prepared from the 18S to 28S RNA of immune-precipitated polysomes (8). In general, the preferential aggregation of any mRNA may be advantageous. In combination with the immune approach developed for the specific isolation of mRNA from fully functional eukaryotic cells (8,9) it would lead to highly purified mRNA preparations.

Two points of interest are mentioned below. Chemical purification of the mRNA from rRNA by passage on oligo(dT)-cellulose do not change its biological purity (Figs. 3 and 4). This is in agreement with the fact that most mRNA species from eukaryotes contain poly(A) segments (12). The enrichment with L-chain mRNA in the 22S as compared to the 12S RNA was hardly reflected in polyacrylamide gel analyses of the cell-free products (Fig. 4), but was evident in fingerprint analyses of the tryptic digests (Fig. 3). Thus, the banding pattern of proteins synthesized in a cell-free system yield little information on the biological purity of the mRNA preparation.

The aggregation of L-chain mRNA may be a general phenomenon since other laboratories reported on mRNA activity in the 19S poly(A)-rich RNA prepared from the phenol extract of total polysome population of MOPC-41 (2) and MOPC-70E (7). Unfortunately, fingerprint analyses of the cell-free products programmed by these mRNAs were not done.

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