DISTRIBUTION OF MOPC-315 LIGHT CHAIN MESSENGER RNA IN FREE AND MEMBRANE-BOUND POLYRIBOSOMES

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SUMMARY: To measure the distribution of immunoglobulin light chain mRNA, polyadenylated mRNA was isolated from membrane-bound polysomes, free polysomes and 100,000 x g supernatant of MOPC-315 plasmacytoma. Each fraction was hybridized with cDNA specific for purified light chain mRNA. Comparison of hybridization kinetics indicated that membrane-bound polysomal mRNA contained approximately three to four times more MOPC-315 light chain mRNA (L^{315} mRNA) per mg of mRNA than the other two fractions. Furthermore, because there are more free than membrane-bound polysomes, membrane-bound polysomes of the MOPC-315 plasmacytoma contain only about twice as much of the L^{315} mRNA as do the free polysomes.

INTRODUCTION

Biosynthesis of immunoglobulin chains was reported to take place predominantly on ribosomes bound to the endoplasmic reticulum (1,2). Based on these observations, in most early studies immunoglobulin mRNA was isolated from membrane-bound polyribosomes. However, we have recently shown that the mRNA fraction derived from membrane-bound polysomes yielded less immunoglobulin mRNA than mRNA derived from total cellular RNA as determined by a translational assay (3,4). As a quantitative assay of mRNA, cell-free translation depends on the relative translational efficiency of various mRNA templates and requires the functional integrity of the mRNA. Thus, as an alternative, we have used molecular hybridization with cDNA to quantitate the distribution of light chain mRNA (L^{315} mRNA) of the MOPC-315 plasmacytoma. The results are presented in this communication.

MATERIALS AND METHODS

Isolation of mRNA. Membrane-bound polysomes, free polysomes and 100,000 x g supernatant fraction (S-100) of the MOPC-315 plasmacytoma were isolated by methods described previously (3). In brief, tumor tissue was homogenized in a loose fitting teflon-glass homogenizer. After centrifugation at 20,000 x g for 20 min, the supernatant was layered over a discontinuous sucrose gradient

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TABLE 1

RELATIVE DISTRIBUTION OF L^{315} mRNA IN mRNA FRACTIONS

mRNA Fractions	$Crt_{1/2}$	L^{315} mRNA (μg/mg mRNA)	Total mRNA Yield (μg/20 g)	Relative Distribution (%)
Membrane-bound polysomes	0.22	44	110	66
Free Polysomes	0.60	16	140	31
S-100	0.80	12	17	3

$Crt_{1/2}$ values were determined from Fig. 1. The μg of L^{315} mRNA/mg of total mRNA was calculated on the basis of a $Crt_{1/2}$ value of 5×10^{-3} for globin cDNA hybridized to globin mRNA under identical conditions. For purposes of calculation, the size of globin mRNA was taken as 650 nucleotides (9) and the size of immunoglobulin L^{315} mRNA as 1250 nucleotides (3,10) in length.

containing 1.5 M and 2.3 M sucrose. The tubes were centrifuged at 94,000 x g for 16 hours. RNA was prepared from each fraction by use of a phenol extraction method. Poly(A)-containing mRNA was isolated by oligo(dT)-cellulose column chromatography as described (3).

Preparation and purification of cDNA specific for L^{315} mRNA. L^{315} mRNA was prepared and partially purified by zonal sedimentation as described (3). Further purification of L^{315} mRNA was performed by polyacrylamide gel electrophoresis in formamide by minor modifications of the procedure of Duesberg and Vogt (5). This purified L^{315} mRNA was used as a template for the synthesis of cDNA by the method of Kacian and Myers (6). The cDNA was additionally purified by hybridization with total RNA from the mouse MOPC-41 plasmacytoma (a κ-chain producer) and hydroxylapatite chromatography. The cDNA not hybridizing to MOPC-41 RNA was used as a probe specific for L^{315} mRNA.

Hybridization Reaction. Hybridization of [^{3}H]cDNA to mRNA was carried out by the method of Gillespie (7). Approximately 500 cpm of [^{3}H]cDNA specific for L^{315} mRNA was annealed with mRNA fractions at 68°C in 0.4 M sodium phosphate, pH 7.0, for 60 hours. Resistance to S_1 nuclease was used to determine the amount of [^{3}H]cDNA hybridized to mRNA. S_1 nuclease was prepared from α-amylase obtained from Sigma by column chromatography on DEAE-cellulose (8).

RESULTS

Poly(A)-containing mRNA fractions were isolated by oligo(dT)-cellulose chromatography from membrane-bound polysomes, free polysomes and S-100 from 20 g of MOPC-315 plasmacytoma. The total amount of mRNA obtained from each fraction was 110, 140 and 17 μg, respectively (Table 1).

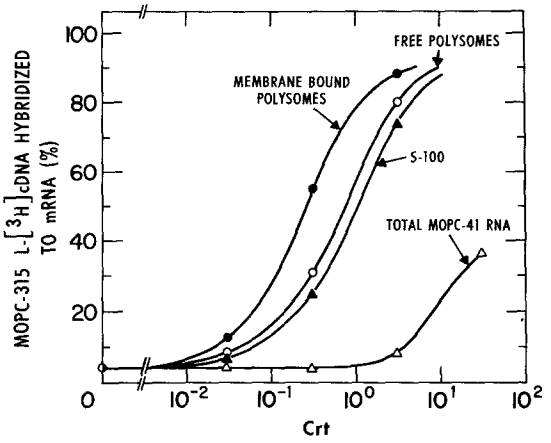

Fig. 1. Hybridization of cDNA specific for L^{315} mRNA with poly(A)-containing mRNA.

Each fraction was annealed to cDNA specific for L^{315} mRNA. From the kinetics of hybridization, the $Crt_{1/2}$ values for the mRNA fractions from membrane-bound polysomes, free polysomes and S-100 were estimated to be 0.22, 0.60, and 0.80, respectively (Fig. 1, Table 1). These results indicated that the membrane-bound polysomal mRNA contained 2.7 and 3.7 times as much L^{315} mRNA per mg of mRNA as did the latter two fractions. Furthermore, by calculating the total amount of L^{315} mRNA in each fraction, it was determined that membrane-bound polysomes, free polysomes, and S-100 fraction contain 66%, 31%, and 3% of the L^{315} mRNA, respectively (Table 1).

DISCUSSION

Our previous results revealed that mRNA derived from membrane-bound polysomes resulted in a lower yield of immunoglobulin mRNA than obtained from total cellular RNA (3). Our present DNA-RNA hybridization experiments indicate that free polysomes contain about one-third of the L^{315} mRNA present in the MOPC-315 plasmacytoma. This result is in agreement with our previous conclusions from translational studies. Why so much L^{315} mRNA remains unassociated with membrane is not clear.

Lisowska-Bernstein et al. (11) have reported that both free and membrane-

bound polysomes isolated from MOPC-70A mouse plasmacytomas synthesize heavy and light immunoglobulin chains. However, Cioli and Lennox (1) suggested such results might be due to possible contamination of membrane-bound polysomes in the free polysome pellet obtained through 2 M sucrose. They also reported that free polysomes obtained through 2.3 M sucrose contained little membrane contamination and found that neither H nor L chains were detectable on the free polysomes from MOPC-21 plasmacytomas (1). Also, Blobel and Dobberstein (2) reported that free polysomes from MOPC-41 plasmacytomas did not contain any detectable light chain mRNA activity. In both cases, however, the existence of some functional light chain mRNA in free polysomes could not be excluded. Our results indicate that free polysomes obtained by sedimentation through 2.3 M sucrose contained approximately one-third of the total cellular L^{315} mRNA. Analogously, Shafritz (12,13) reported that, although albumin is translated by membrane-bound polyribosomes of rat liver, both free and membrane-bound polysomes contain functional albumin mRNA. Thus, a relatively large amount of mRNA specific for secretory proteins appears in the free polyribosome fraction of rat liver. This mRNA is translated on membranes and appears to be in an inactive form on the free polysomes. Although this fraction represents about 30% of the total L^{315} mRNA, it conceivably may be a transitory component on the pathway to association with membranes. Possibly the large amount of immunoglobulin mRNA synthesized overwhelms the capacity of these cells to translate all the L^{315} mRNA present on the membranes of the plasmacytomas. Nevertheless, the generalization that immunoglobulin mRNA is to be found exclusively on membrane-bound polyribosomes is undoubtedly wrong. This is particularly noteworthy for the preparation of immunoglobulin mRNA. In any case, use of total mRNA instead of membrane-bound mRNA simplifies the preparative procedures and results in a greater yield of immunoglobulin mRNAs from the MOPC-315 plasmacytoma and, probably, other plasmacytomas as well.

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