ELECTROPHORETICALLY HOMOGENEOUS MYELOMA LIGHT CHAIN mRNA AND ITS TRANSLATION IN VITRO

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SUMMARY. mRNA coding for the light chain of a myeloma protein has been purified to give one band in acrylamide gel electrophoresis. This pure RNA (S~13.5) could be translated *in vitro* into the light chain in a heterologous cell-free translation system. The light chain synthesized *in vitro* is apparently slightly larger than the light chain secreted by the tumor.

mRNA coding for light chains of myeloma proteins has been extracted from mouse plasma cell tumors and successfully translated in several systems; reticulocyte lysates (1,2), a cell-free system of Ascites tumor cells (3,4), and *Xenopus* eggs (5). But only a partial purification of such mRNA has so far been reported. It would be essential to obtain immunoglobulin mRNA of high purity for important experiments, such as sequence studies and DNA-RNA hybridization. Data from hybridization studies should enable us to choose between the two current theories for the generation of antibody diversity; germ line genes vs. somatic variability (see e.g. 6). Since some myeloma cell lines are adapted to tissue culture, it is possible to label mRNA with ^{32}P at sufficiently high specific activities to carry out some of these studies. In this communication we describe the procedure for the purification of ^{32}P labelled light chain mRNA to a state of electrophoretical homogeneity.

The mRNA was isolated from murine plasma cell tumor MOPC 70E forming only κ light chains. This tumor had been adapted to suspension tissue culture and kindly provided by Dr. Ostertag of the Max Planck Institute in Göttingen. The cells labelled with ^{32}P-phosphate for 9 hr were harvested and disrupted in a glass homogenizer. Free polysomes (FP) and membrane-bound polysomes (MBP) were isolated and purified by discontinuous sucrose gradient centrifugation (7). RNA was extracted from polysomes with phenol and was fractionated by poly(U)-cellulose column chromatography

essentially by the method of Sheldon et al. (8). With either FP RNA or MBP RNA, 2% to 3% of the input material was bound to the column and eluted at 45°C at low ionic strength. The mRNA activity of the column fractions was assayed in a partially purified cell-free translation system developed by M. Schreier and T. Staehelin in this Institute (9). With both FP and MBP RNA the fractions retained by the column stimulated incorporation of radioactive amino acids into proteins at least five times more efficiently than the unretained fractions.

From analyses of the proteins synthesized endogenously by isolated free and membrane bound polysomes (10), it has been suggested that the light chain mRNA is mostly in MBP. In order to test directly the validity of this suggestion we subjected the protein products directed by RNA isolated from FP and MBP to sodium dodecylsulfate (SDS)-acrylamide gel electrophoresis. Figure 1 shows the electrophoretic analysis of the products translated in vitro from the FP 45°C RNA fraction (Figure 1A) and the MBP 45°C RNA fraction (Figure 1B) after precipitation with rabbit anti-mouse κ-chain serum (specific precipitate). The preparation of rabbit reticulocyte initiation factors used in this experiment contained an unusually large amount of globin mRNA. This is the explanation of the large peak of globin seen in Figure 1; this globin was carried over during immunoprecipitation due to non-specific adsorption. Other than the globin peak, a component which migrated slightly more slowly than the marker light chain can be seen in Figure 1B. Such a component was not observed when normal rabbit serum was used for the immunoprecipitation (control precipitate). Other than this component the profile of the control precipitate was essentially the same as that of the specific precipitate. Furthermore, when exogenous mRNA was not added (background), no such component was observed either in the specific precipitate or in the control precipitate. These observations indicate that this component is clearly related to the light chain in its structure and was translated from the light chain mRNA in the MBP 45°C RNA fraction. A similar light chain related component had been observed in the products synthesized in other cell-free systems (2,3) and has been postulated to be a precursor of the light chain secreted by myeloma cells. In Figure 1A only

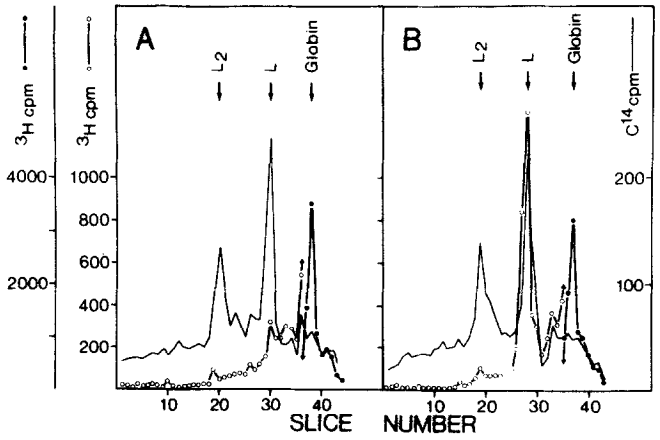

Figure 1: SDS-acrylamide gel electrophoresis of products translated in vitro from poly(U)-cellulose column RNA fractions. The composition of the in vitro assay mixture (final volume 0.1 ml is described in reference 10 except mRNA. The assay mixture contained 2 µg FP RNA (Fig. 1A) or MBP RNA (Fig. 1B), which was retained by the poly(U)-cellulose column at 4°C with a high ionic strength buffer (0.01M Tris-HCl pH 7.5, 0.15M NaCl) and eluted at 45°C with a low ionic strength buffer (0.01M Tris-HCl pH 7.5). After 50 min incubation at 30°C, an aliquot of the reaction mixture was mixed with 5 µl of rabbit anti-mouse κ-chain serum. After 15 min incubation at 37°C, 50 µl of sheep anti rabbit γ-glonulin serum was added and the mixture was incubated at 37°C for 20 min, after which the mixture was allowed to stand at 4°C for 12 hr. Precipitate was collected, washed with a phosphate-buffered saline, and subjected to acrylamide gel electrophoresis according to the procedure described before (14). The light chain marker is the medium in which 70E cells have been grown overnight in the presence of (^{14}C)-leucine. L = light chain, L_2 = dimer of light chain. ^{3}H cpm = in vitro products labeled with (^{3}H)-leucine, ^{14}C cpm = light chain markers.

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trace amounts of such a component was observed, confirming the view that the free polysomes contain very little, if any, light chain mRNA. Since poly(U)-cellulose columns retain only mRNA with a poly(A) sequence greater than a certain length, one might argue that FP contain light chain mRNA without such a poly(A) sequence. In order to test this possibility FP RNA was fractionated by sucrose gradient centrifugation in a zonal rotor and fractions corresponding to 17-15S, 14-12S, and 11-7S were separately tested for light chain mRNA activity by the procedures described above. No appreciable activity was observed in any of the three RNA fractions.

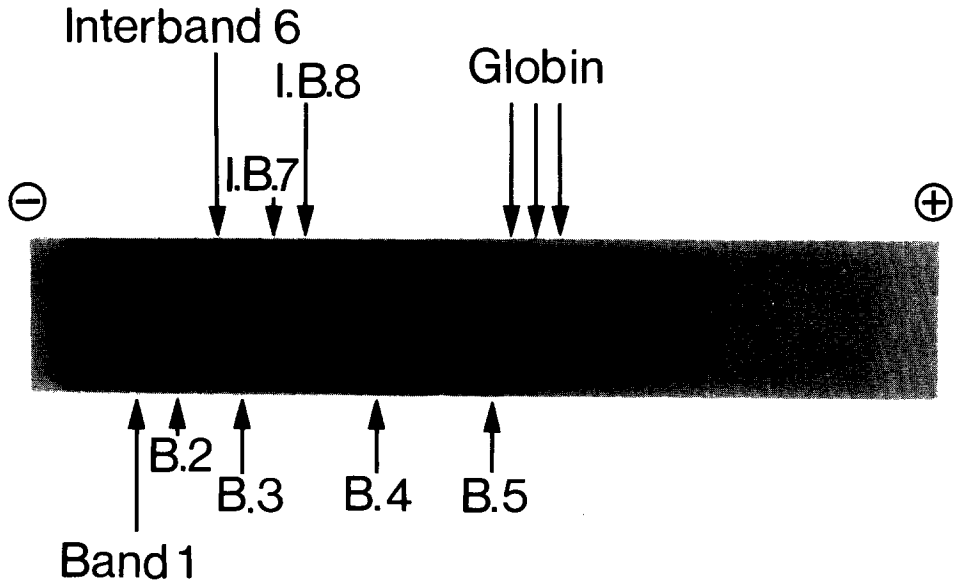

Figure 2: Autoradiograph of preparative acrylamide gel electrophoresis of MBP 15-12S RNA. 4 A_{260} units of MBP 15-12S RNA dissolved in 150 µl of 0.01M Tris-HCl pH 7.2 containing 0.01M ethylene diamine tetraacetate was applied to a 4.7% acrylamide gel cast in an E.C. apparatus (Milton Roy Co., Florida). Electrophoresis was carried out at 9°C at 200 volts for 21 hr. Autoradiography of the wet slab gel was carried out following the procedure of Adams et al. (15). The positions of rabbit globin mRNA (a generous gift of Dr. T. Staehelin) were determined by a parallel run.

The fraction of MBP RNA retained by the poly(U)-cellulose column was further fractionated by sucrose density gradient centrifugation. The fractions corresponding to 19-16S, 15-12S, and 11-7S were separately pooled and assayed for light chain mRNA activity in the in vitro system. More than 90% of the light chain mRNA activity was in the 15-12S fraction, the rest being in the 19-16S fraction.

The 15-12S fraction was further fractionated by preparative slab polyacrylamide gel electrophoresis following the procedures described by Peacock and Dingman (11). Figure 2 shows the autoradiograph prepared from the slab gel. Because the gel is 3 mm thick, the quality of the autoradiograph is not expected to be as good as the conventional ones which are usually made on dried

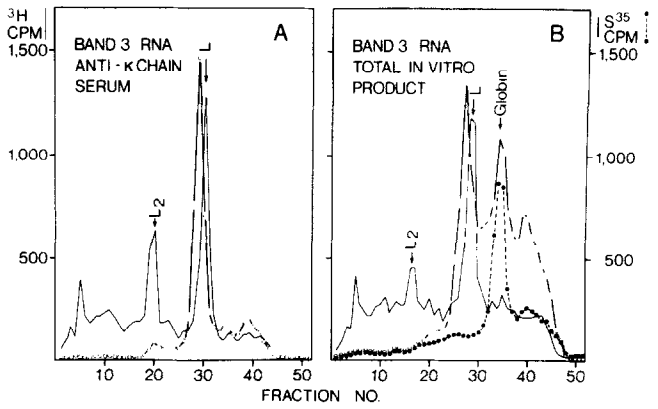

Figure 3: SDS-acrylamide gel electrophoresis of products translated in vitro from band 3 RNA. Band 3 region of the gel was cut out from the slab using the autoradiograph as a guide. The gel was broken into small pieces in a loose fitting glass homogenizer. RNA was extracted by washing the crushed gel 5 times with 0.5M Na-acetate. The procedures of in vitro translation and analysis of the products are as described in the legend to Fig. 1. except that (^{35}S)-methionine was used to label the products and (^{3}H)-leucine to prepare the light chain markers. o———o with band 3 RNA, •-----• without band 3 RNA (background products).

gels. Band 1 is 18S ribosomal RNA. Between the positions of 18S ribosomal RNA and the marker globin mRNA, two major bands (bands 2 and 3) and two minor bands (bands 4 and 5) are observed. RNA in these bands and in the regions between the bands (indicated in Figure 2) was eluted and assayed for mRNA activity in the in vitro system. All RNA fractions, except that from band 1, exhibited roughly the same stimulatory activity per microgram of RNA. The in vitro products were precipitated by anti κ-chain serum and the precipitate was analyzed by SDS-acrylamide gel electrophoresis. Among the RNA fractions eluted from the five bands (bands 1 to 5), only band 3 RNA promoted the synthesis of the light chain-related component. In Figure 3B the profiles of the SDS-acrylamide gel electrophoresis of the whole in vitro products translated from band 3 RNA is shown. Approximately 40% of the in vitro product migrated as the component (MW = 25,000) slightly larger than the marker light chain (MW = 23,500). This component is quantitatively precipitated by an anti κ-chain serum (Figure 3A). Roughly 20% migrated as a sharp band at the position (MW = 18,000) corresponding to globin. Since the background products also contain this component in nearly the same amount, most of the 18,000 MW

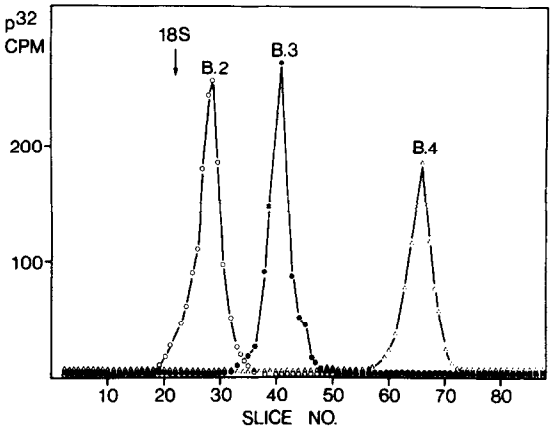

Figure 4: Re-electrophoresis of RNA eluted from acrylamide gel slab. The procedures of extraction and electrophoresis of the RNA are as described in the legends to Fig. 3 and Fig. 2 respectively.

component is thought to be translated from the endogenous globin mRNA. The rest migrated heterogeneously between the light chain marker and the anode end of the gel. Although these products may be partially due to contaminating mRNA in the band 3 RNA, they could also have arisen by false initiation or more likely by premature termination of translation of light chain mRNA. RNA eluted from the regions immediately adjacent to band 3 also promoted the synthesis of the light chain-related component but to a lesser extent. The re-electrophoresis of the RNA's from these regions revealed that they were contaminated by band 3 RNA.

Figure 4 shows the results of re-electrophoresis of the RNA eluted from some of the bands. Band 3 RNA migrated, as a sharp band, 2.5 times faster than 18S ribosomal RNA and 2.9 times slower than rabbit globin mRNA. The same RNA sedimented in sucrose gradient centrifugation as a sharp band at a position of 13.5S. These results indicate that our light chain mRNA preparation is of similar purity as other eucaryotic mRNA so far isolated and successfully used in DNA-RNA hybridization studies (12,13).

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