# TRANSLATION OF MESSENGER RNA FOR IMMUNOGLOBULIN LIGHT CHAINS IN A CELL-FREE SYSTEM FROM KREBS II ASCITES CELLS

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## 1. Introduction

Attempts to isolate the messenger RNA (mRNA) for immunoglobulin light (L) chains have been hampered by the lack of a reliable assay for the mRNA. Recently, however, the synthesis of a mouse light chain in a cell-free system from rabbit reticulocytes was reported [1]. We have employed an alternative heterologous assay system, derived from Krebs II ascites cells, to identify the mRNA. Previous work has shown that both globin and lens mRNAs are faithfully translated in this system [2, 3]. As a source of the mRNA we chose the mouse tumour MOPC 21 engaged in the production of an IgGl ($\kappa$) immunoglobulin [4]. The light chain of this molecule has been fully sequenced [5, 6]. We show here that an RNA fraction from this myeloma directs the synthesis in the ascites system of MOPC 21 light chains.

## 2. Methods

[$^{35}$S] methionine-labelled and carrier, unlabelled, MOPC 21 L-chain were prepared essentially as before [7]. RNA was extracted from solid tumours as in [1] but in the presence of 3% diethylpyrocarbonate. It was fractionated on a 650 ml linear 10–30% sucrose gradient in 0.1 M NaCl, 0.005 M EDTA, 0.01 M Tris chloride, pH 7.5 for 18 hr at 28° at 40,000 rpm in an MSE B XIV zonal rotor using 50 ml of overlay. Fractions were made 0.2 M with respect to KCl and potassium dodecylsulphate was removed by centrifugation at 4°. RNA was precipitated with 2 vol ethanol. The precipitate was redissolved in 0.1 M KCl, reprecipitated and washed with 75% ethanol, 100% ethanol and dried *in vacuo*.

The 30,000 *g* supernatant (S-30) of Krebs II ascites cells was prepared, preincubated and used as described previously [2]. Aliquots from the incubation mixture were removed for estimation of total protein synthesis by precipitation with trichloroacetic acid (TCA), and the remainder was treated with pancreatic RNase (0.1 mg/ml for 15 min at 37°) and analysed by electrophoresis on 12½% SDS-polyacrylamide gels [2]. The stained gels were sliced longitudinally, dried and autoradiographed. Protein was eluted from gel bands in the presence of 1 mg carrier MOPC 21 L-chain with 1% SDS, recovered by precipitation with TCA (final conc. 10%), washed with acidified acetone and dried *in vacuo*. It was oxidized with performic acid and digested in 1% ammonium bicarbonate overnight at 37° with 20 $\mu$g of trypsin. The digest was analysed as described below.

## 3. Results

### 3.1. *Preparation of mRNA*

Fig. 1(a) shows the large-scale preparation of messenger RNA from microsomes by sucrose density gradient centrifugation. The conditions of run were such as to pellet the 18 S rRNA thus allowing maximal separation in the region (approx. 10–12 S) where the mRNA from light chains was expected [1]. In addition, about 2% of the preparation was analysed as shown in fig. 1(b), and this profile did not appear to show a significant amount of ribosomal RNA breakdown.



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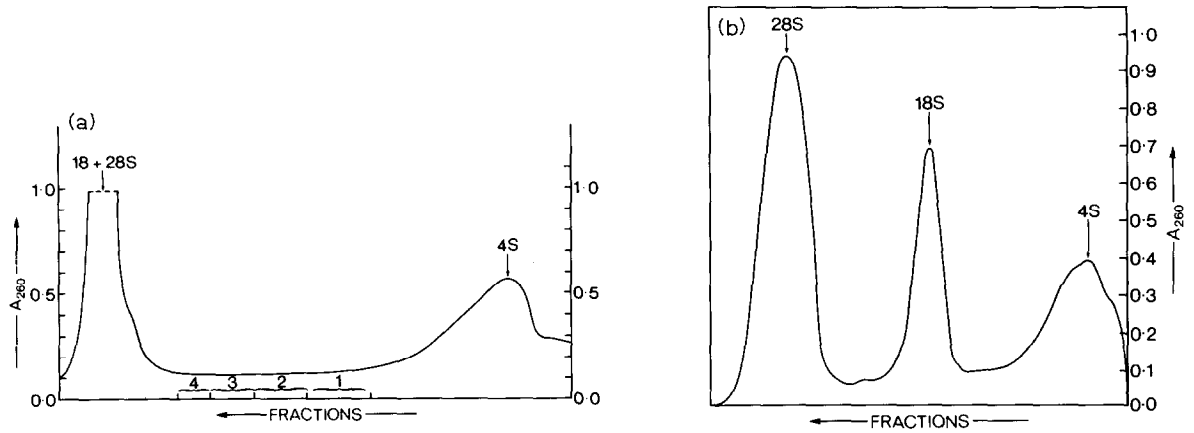


Fig. 1. Fractionation of RNA from microsomes by sucrose density gradient centrifugation. (a) Preparative fractionation on a zonal rotor. (b) Analytical fractionation on a swinging-bucket rotor.

### 3.2. *Cell-free synthesis*

Fractions from the preparative sucrose gradient (fig. 1a) were tested in the ascites cell-free system. Fig. 2 shows that there was a 3-fold stimulation of protein synthesis when fractions 2 and 3 were assayed. The stimulation was optimal at an RNA concentration of about 3–5 $A_{260}$ units/ml and declined at higher concentrations. Fraction 1 was about equally active. The $^{35}S$-labelled cell-free products were analysed by SDS-acrylamide gel electrophoresis (fig. 3). One of the two prominent radioactive components present in the experimental gels and absent from the control co-electrophoresed with unlabelled marker L-chains, added before the electrophoresis. Fractions 2 and 3 gave similar results. Cell-free synthesis of radioactive light chains was also obtained when the input label was a mixture of $^{14}C$-labelled amino acids instead of [$^{35}S$] methionine. Fraction 1 and 18 S rRNA were also tested, and in neither case was there a band in the region of the L-chain, although fraction 1 was active in stimulating protein synthesis.

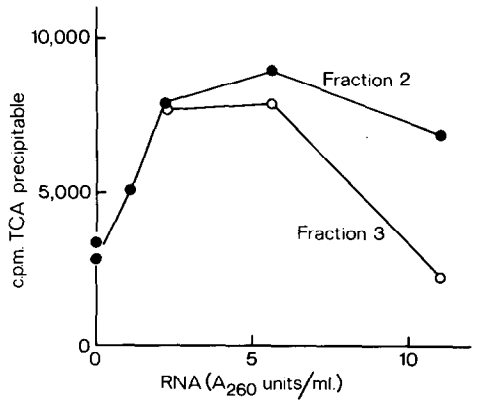


Fig. 2. Stimulation of protein synthesis by myeloma RNA fractions. Incorporation of mixed $^{14}C$-labelled amino acids into hot TCA-insoluble material was measured as previously [3].

### 3.3. *Fingerprint analysis*

The light chains of MOPC 21 IgG contain 4 methionine residues at positions 4, 11, 13 and 175. Met 4 occurs in the N-terminal (basic) tryptic peptide T1: Asn–Ile–Val–Met–Thr–Gln–Ser–Pro–Lys (residues 1–9) while Met 11 and 13 occur together in the next (neutral) tryptic peptide, T2: Ser–Met–Ser–Met–Ser–Val–Gly–Glu–Arg (residues 10–18). Met 175 occurs in a tryptic peptide, T16: Asp–Ser–Thr–Tyr–Ser–Met–Ser–Ser–Thr–Leu–Thr–Leu–Thr–Lys. Fig. 4 shows a fingerprint of [$^{35}S$] methionine-labelled tryptic peptides obtained from purified light chains labelled in tissue culture.

To confirm that the material synthesized *in vitro* by the ascites system was indeed MOPC 21 light chain, we analysed the tryptic peptides of the pooled content of the gel band in the position of light chain (see fig. 3) from 10 individual gels. After electrophoresis at pH 6.5 a faint band was observed in the

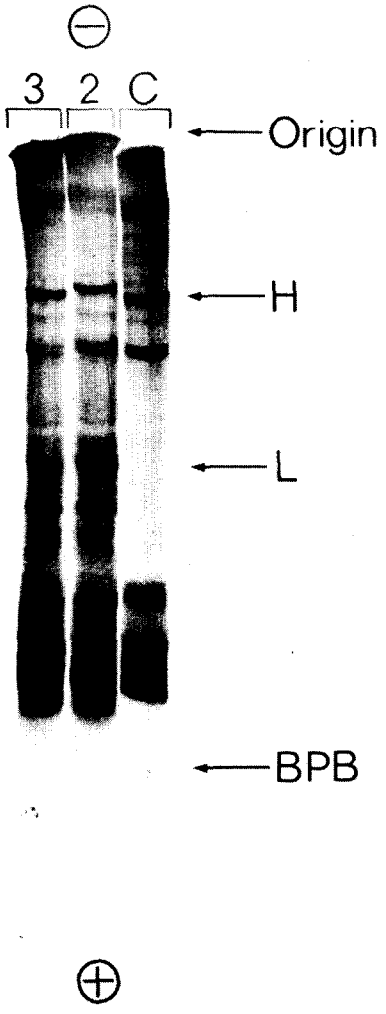


Fig. 3. Analysis by SDS acrylamide gel electrophoresis of the [$^{35}$S]-labelled cell-free products. C: no added RNA; 2 and 3: fractions 2 and 3 respectively, each at 2.8 $A_{260}$ units/ml final concentration. L and H mark the position of unlabelled L and H chains respectively, PBP the bromphenol blue.

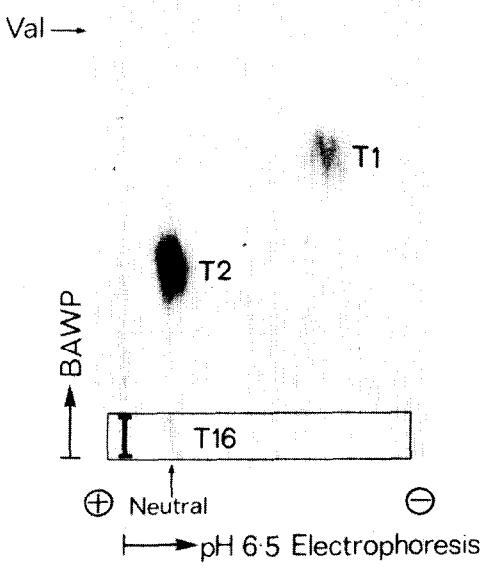


Fig. 4. Radioautograph of a peptide map of [$^{35}$S]methionine-labelled MOPC 21 L-chains. Peptides T1, T2 and T16 are identified in the text. Val, a valine marker in the chromatographic dimension.

basic region having the same mobility as the marker T1, as well as a stronger neutral band in the position of T2 and T16. Other (fainter) bands were also observed but were not studied further. The neutral and basic bands were chromatographed in the second dimension in parallel with T1 and T2 markers. Fig. 5 shows that the major component of the neutral band had an $R_f$ identical to the T2 marker (which was contaminated with other minor components). The main component of the basic band also coincided with marker T1. As carrier light chain had been added before tryptic digestion it was possible to show that the radioactivity in cell-free products T1 and T2 exactly corresponded to the ninhydrin spots known to be authentic T1 and T2. Peptide T16 which occurs in low yield (see fig. 4) was not observed in the cell-free product.

## 4. Discussion

These results are a clear indication of cell-free synthesis of MOPC 21 light chains in the ascites system. However, this synthesis was rather inefficient in comparison with the template activity of mRNA from other sources [2, 3], and it was difficult to obtain sufficient $^{35}$S-labelled product for the fingerprint analysis.

The fact that mRNA directs the synthesis of the

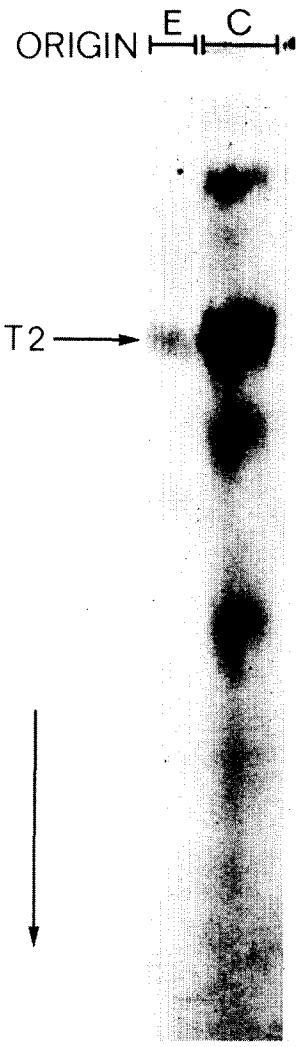


Fig. 5. Descending chromatography of [$^{35}$S]methionine-labelled peptides. Solvent was butanol:acetic acid:water:pyridine, 15:3:12:10 by vol. E: cell-free labelled neutral peptides; C: marker T2 (contaminated with other minor components).

entire light chain in heterologous systems (see also [1 and 8]) supports the idea that both variable and constant regions of light chains are translated from a single RNA molecule. However, final proof for this will only be entirely convincing when the mRNA sequence becomes available.

In more recent experiments we have observed the synthesis of another component, with slower mobility than light chains, on SDS-gel electrophoresis. This appears to be a precursor of light chains and will be the subject of a separate communication.

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