THE SYNTHESIS OF LIGHT CHAIN IMMUNOGLOBULIN IN MPC-11 CELLS ON ONLY ONE OF TWO FRACTIONS OF MEMBRANE-BOUND POLYSOMES PREPARED BY A COMBINATION OF NITROGEN CAVITATION/ DETERGENT TREATMENT

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1. Introduction

Membrane-bound polysomes prepared from myeloma cells disrupted by Dounce homogenisation were an underestimate of the true proportion of bound polysomes since the nuclear pellet also contained polysomes [1]. Nuclei of MPC-11 cells isolated after disruption by nitrogen cavitation were seen to be surrounded by strands of cytoplasmic material [2] which was presumed to be endoplasmic reticulum. In the work reported here it is shown that about 50% of total membrane-bound polysomes are released on endoplasmic reticulum when cells are disrupted by nitrogen cavitation (fraction 1) whilst the remainder are bound to endoplasmic reticulum still attached to the nucleus and were only released upon detergent treatment of the nuclei (fraction 2). The synthesis of light chain immunoglobulin was shown to occur almost exclusively on polysomes in fraction 1.

2. Materials and methods

2.1. *Cell line and growth conditions*

A mouse plasmacytoma cell line (MPC-11) which produces only light chain immunoglobulin was grown in suspension culture as previously described [2].

2.2. *Disruption of cells and isolation of membrane-bound polysomes*

Cells were disrupted by the technique of nitrogen caviation [3]. About 2.5×10^8 cells were suspended in 3 ml homogenisation buffer (0.12 M KCl, 0.12 M RNAase-free sucrose, 7.5 mM $MgCl_2$, 0.5 mM $CaCl_2$ and 20 mM Tris pH 7.4) and equilibrated with nitrogen at 600 p.s.i. for 20 min in a precooled nitrogen cavitation bomb (Artisan Industries, Waltham, Mass.). Cells were ruptured on sudden release of pressure. The homogenate was centrifuged at 800 *g* for 5 min to pellet nuclei and the supernatant was then centrifuged at 27 000 *g* for 10 min to obtain a membrane pellet. Both the nuclear and membrane pellets were washed with buffer and resuspended in buffer containing 0.2% Kyro EOB (a non-ionic detergent, see [2]) and 0.2% sodium deoxycholate (DOC). After gentle shaking for several minutes at 0°C, nuclei and unsolubilized material were removed from the respective preparations by centrifugation at 7500 *g* for 5 min.

As a result of nitrogen cavitation less than 0.1% of cells remained intact. The recoveries of polysomes, determined from absorption at 260 nm, in fractions 1 and 2 were about 95% and 90%, respectively.

2.3. *Sucrose density gradient centrifugation*

Polysomes were separated into size classes as previously described [2]. After piercing the tubes at

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the bottom, the absorption at 260 nm of the contents was monitored in a flow cell mounted in a Gilford Model 242 spectrophotometer.

2.4. *Labeling of cells and release of radioactive nascent polypeptides from polysomes*

About 2 X 10^8 MPC-11 cells were washed with 100 ml culture medium diluted 1:20 with balanced salt solution and were then resuspended in 5ml of the same diluted medium. Incubation was performed at 37°C for 15 min and then 6 μCi U-C^{14}- labeled amino acids (high specificity mixture, code CFB 104, The Radiochemical Centre, Amersham) were added. After a further incubation for 3 min an equal volume of ice-cold medium containing 5 mM sodium azide was added. The cells were washed twice and resuspended in homogenisation buffer. Cells were disrupted by nitrogen cavitation and the two fractions of membrane-bound polysomes isolated as described above. Both polysome fractions were made up to 0.75 M in KCl and puromycin was added to a final concentration of 1.6 μg/ml. After a 15 min incubation at room temperature each was centrifuged at 220 000 *g* for 3 hr to remove ribosomal subunits and remaining undissociated polysomes.

2.5. *Isolation of labeled light chain polypeptides*

Aliquots of each high speed supernatant from the two polysome fractions were immediately taken for immunoprecipitation and light chain was assayed as previously described [4]. Similar aliquots were precipitated with 10% trichloroacetic acid (TCA) and the total incorporation of radioactivity into polypeptides in each fraction was determined.

3. Results and discussion

We have previously reported that only free polysomes are released from MPC-11 and L cells as a result of treatment with the non-ionic detergent Kyro EOB [2]. Membrane-bound polysomes were released only when DOC was added and these polysomes were shown to be active in the synthesis of immunoglobulin light chain [4]. In both cell lines total polysomes were almost equally divided into the free and membrane-bound classes. Since Kimmel [1], using Dounce homogenisation, had observed that not all bound polysomes were released and the fact that a similar observation was made following nitrogen cavitation [2], it was decided to solubilize those polysomes which remained on endoplasmic reticulum bound to the nucleus in order to ascertain the relative distribution of total membrane-bound polysomes and in addition investigate a possible difference in function.

Total membrane-bound polysomes were separated into two fractions as described in Materials and methods. The absorption profiles are shown in fig. 1. Approximately 50% of membrane-bound polysomes

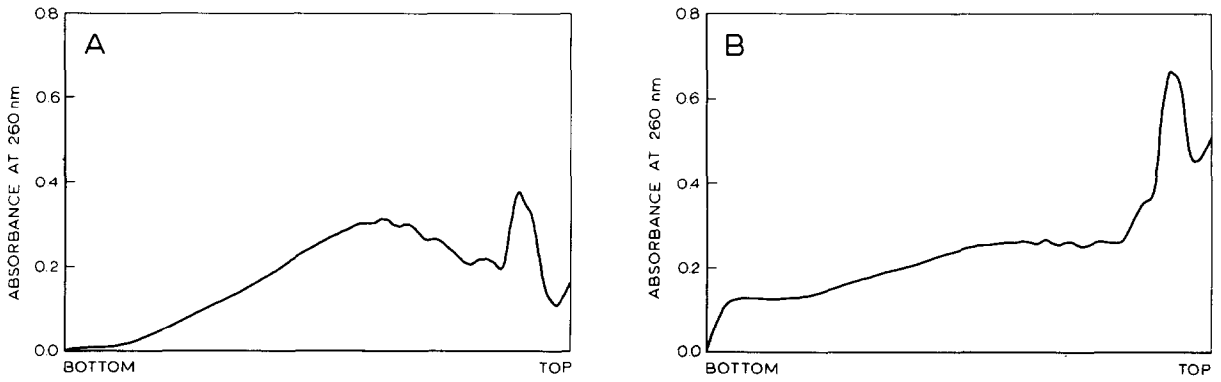

Fig. 1. Polysome profiles of two fractions of MPC-11 membrane-bound polysomes. Total membrane-bound polysomes from MPC-11 cells were separated into two fractions and polysome profiles obtained as described in Materials and methods. A. Fraction 1: polysomes released on endoplasmic reticulum by nitrogen cavitation. B. Fraction 2: polysomes bound to endoplasmic reticulum which remained attached to the nucleus.

were released on membranes by nitrogen cavitation whilst the remainder were bound to endoplasmic reticulum attached to the nucleus. When polysomes were to be solubilized from the nuclear fraction it was necessary to add Kyro EOB in addition to DOC since the latter alone caused complete rupture of nuclei. Kyro EOB was apparently able to protect the nuclei against rupture by DOC.

In control experiments the 15 min incubation in 1:20 diluted medium was found to have no significant effect on the polysome profiles of either fraction. Polysomes in both fractions were disrupted to the same extent by the KCl/puromycin treatment, hence there was no difference in sensitivity in this respect.

The two fractions of membrane-bound polysomes isolated here presumably have different intracellular localisations since those bound to membranes released by nitrogen cavitation (fraction 1) were most likely situated further from the nucleus than those polysomes in fraction 2. To determine whether these two populations of membrane-bound polysomes were merely subfractions of the same homogeneous polysome class, or in fact represented a differential separation of membrane-bound polysomes into two distinct fractions possessing individual functions, it was decided to utilise a known property of myeloma membrane-bound polysomes. In a previous paper [4] we demonstrated that light chain synthesis in MPC-11 cells occurs on membrane-bound polysomes. In these experiments total membrane-bound polysomes were used. The respective abilities of the two fractions of membrane-bound polysomes isolated as described here to synthesise light chain was therefore tested.

MPC-11 cells were labelled with [C^{14}] amino acids for 3 min and the two fractions of membrane-bound polysomes were isolated as described in Materials and methods. Polysomes were dissociated and nascent polypeptide chains were released by incubation in the presence of KCl and puromycin. After the removal of subunits and undissociated polysomes by high speed centrifugation, the supernatants were immunoprecipitated using a sandwich technique and the incorporation of radioactive amino acids into light chain was ascertained. The results, which are shown in table 1, demonstrate that light chain is primarily synthesised in fraction 1 of total membrane-bound polysomes. The polysomes on endoplasmic reticulum not released from the nucleus were not apparently engaged to the same extent in the synthesis of light chain. Fraction 2 polysomes, however, were somewhat more active in protein synthesis.

Table 1
The synthesis of light chain by two fractions of membrane-bound polysomes from MPC-11 cells.

Membrane-bound polysomes	TCA Precipitated cpm/mg protein	Light chain cpm/mg protein
Fraction 1	2140	1730
Fraction 2	3100	350

MPC-11 cells were labeled with [C^{14}]amino acids for 3 min and two fractions of membrane-bound polysomes were isolated as described in Materials and methods. Polysomes were dissociated by high salt and puromycin treatment. Following the removal of subunits etc. by high speed centrifugation, aliquots were assayed for light chain and the incorporation of radioactivity into TCA precipitated material was determined in each fraction.

These findings indicate that there is a difference in function between the two fractions of membrane-bound polysomes and it must be assumed, therefore, that the separation is not merely an experimental artifact. It has been suggested that membrane proteins are synthesised on membrane-bound polysomes [5,6] and the sequence of events in membrane synthesis is most likely as follows: rough endoplasmic reticulum → smooth endoplasmic reticulum → Golgi apparatus → vesicles → plasma membrane [7]. It is possible that active synthesis of new endoplasmic reticulum occurs in the neighbourhood of its proximity with the nucleus and it is thus tempting to suggest that those membrane-bound polysomes in fraction 2 which remain on endoplasmic reticulum attached to the nucleus are those concerned with the synthesis of the basal elements of the endoplasmic reticulum

The results described here indicate a further compartmentalization of the mRNA which codes for light chain. This raises interesting questions concerning how a mRNA-containing complex is able to recognize a specific region of membrane to which it is to bind. Two possibilities would seem likely.

Firstly, recent observations indicate that a precursor form of light chain is synthesised in some cell-free systems [8–11] and the highly hydrophobic N-terminal amino acid sequence [11] may well have a recognition site in the membrane as suggested by Baglioni and Liberti [12], ultimately causing the mRNA–ribosome complex to become membrane bound. Secondly, a specific protein of the informosomal particle may have a particular affinity for a binding site on a membrane component and the particle would thus be predestined to bind to the appropriate membrane region, The mRNA to be then translated by membrane-bound ribosomes. The correct explanation must await further experimentation.

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