THE STIMULATION OF LIGHT CHAIN IMMUNOGLOBULIN SYNTHESIS IN VITRO BY 0.5M SALT WASH OF MPC-11 MEMBRANE-BOUND POLYSOMES ISOLATED IN THE G1 AND G2 PHASES OF THE CELL CYCLE.

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Summary. In vitro polypeptide synthesis using a combination of G1 membrane-bound polysomes and either G1 or G2 0.5M salt wash gave appreciable incorporation into light chain immunoglobulin. When G2 polysomes were used with G2 salt wash, light chain synthesis was much reduced, however, when G2 salt wash was replaced by that from G1 then the synthesis of light chain by G2 polysomes was stimulated. The results suggest that some factor present in the G1 phase was able to activate translation of light chain mRNA which is apparently quiescent in the G2 phase.

The synthesis of immunoglobulin has been shown to be most active toward the end of G1 and the beginning of S phase (1-4). Restriction of the synthesis of light chain immunoglobulin to a certain phase of the cell cycle could be explained either by breakdown of light chain mRNA after the period of active translation, by loss of a specific effector molecule from the informosome or readdition of a 'locking factor' as suggested by Spirin (5). Recent estimates of the half-life of poly (A)-containing mRNA in cells that proliferate rapidly suggest that it turns over perhaps once per cell generation and this would argue against the possibility that breakdown of mRNA alone is the most important factor in the control of protein synthesis. The mRNA which codes for light chain is indeed long lived (6,7) and furthermore Cowan and Milstein (7) suggest that the mRNA must survive mitosis, presumably to be translated in the following cell cycle. This suggestion is substantiated by recent observations in our laboratory where cells in the G2 phase have been shown to synthesise only small amounts of light chain but immediately after the completion of mitosis synthesis commences rapidly (to be published).

We have reported the existence of a factor in the 0.5M salt wash of membrane-bound polysomes in MPC-11 cells which possesses specificity for this class of polysomes (8). Since the synthesis of light chain in MPC-11 cells is known to occur on membrane-bound polysomes (9) it is tempting to speculate that the above observation may indicate the presence of a specific factor for the efficient translation of light chain mRNA. The requirement of specific initiation factors for the effective translation of several mRNAs is now well established (10-14).

It is evident that the light chain mRNA although present in the cell during all phases of the cell cycle is not translated at the same continuous rate in the various phases. The knowledge that the mRNA is long lived strongly suggests that regulation must be at the level of some specific factor.

Experimental.

Cell line and growth conditions. MPC-11 cells (15) were grown in Dulbecco modified Eagle medium containing in addition per 10 liters : 37g sodium bicarbonate, 5.8g glutamine and 1g sodium pyruvate. The pH was finally adjusted to 7.2. In addition the medium contained 15% heat inactivated horse serum and 25U penicillin and 25µg streptomycin per ml.

Synchronization of cell cultures. Cells were synchronized by growth in isoleucine deficient medium (4,16,17) for 20-25 hrs before transfer to complete medium. Cultures were harvested either at the end of G1 phase (2 hrs after transfer) or in G2 phase (12 hrs after transfer).

Isolation of membrane-bound polysomes and preparation of 0.5M salt wash. 3.10^8 cells were suspended in 4ml buffer A (100mM KCl, 5mM magnesium acetate, 20mM Tris pH 7.6) and homogenized using the technique of nitrogen cavitation (18,19). The post-mitochondrial supernatant was centrifuged at 27000xg for 10 min to give a microsomal pellet containing the membrane-bound polysomes which synthesise light chain immunoglobulin (19). The pellet was resuspended in buffer A and solubilized by the addition of a mixture of Kyro EOB (a non-ionic detergent) and sodium deoxycholate (15). Polysomes were pelleted by centrifug-

ation at 150,000xg for 2 hrs in the 460 rotor of the IEC B-60 ultracentrifuge. The pellet was resuspended gently in buffer A and 4M KCl was added slowly with mixing to a final concentration of 0.5M. Following incubation at 0° for 30 min the polysomes were again pelleted by high speed centrifugation, resuspended in buffer A containing 5% glycerol and stored in small aliquots at -90°. The clear supernatant from the second high speed centrifugation (the 0.5M salt wash of polysomes) was dialysed for 12 hrs against one hundred volumes of buffer A containing 5mM 2-mercaptoethanol and 5% glycerol. After dialysis the salt wash was divided into 100µl aliquots, frozen and stored at -90°.

Polypeptide synthesis in vitro. In vitro synthesis was carried out as described previously (15) except that high speed supernatant from Krebs II ascites cells was used , and pH5 fraction isolated from MPC-11 cells by the method of Falvey and Staehelin (20) replaced mouse liver tRNA. In some assays 0.5M salt wash protein of MPC-11 membrane-bound polysomes was added. Following incubation at 37° reaction mixtures were chilled in ice and diluted with an equal volume of a solution containing 0.025-0.5mM non-radioactive amino acids. Aliquots were immediately taken for immunoprecipitation and radioactivity incorporated into light chain was determined as previously described (9).

Results and discussion.

Membrane-bound polysomes were isolated toward the end of G1 phase where light chain synthesis is appreciable and in the G2 phase where synthesis is minimal. A 0.5M salt wash and corresponding salt washed polysomes were prepared from each population. The abilities of G1 and G2 salt washes to stimulate light chain synthesis in vitro using G1 and G2 salt washed membrane-bound polysomes was tested. The concentration of protein added was that which gave maximal stimulation of light chain synthesis in the G1 and G2 systems respectively. The results are presented in Table 1 and are the average values from three separate experiments. In each experiment a variety of control assays were performed and these demonstrated that only a background of 70-85 cpm was achieved when the in vitro mixture did not

Table 1. Incorporation of [^{14}C]-labeled amino acids into light chain in vitro.

Additions to the in vitro mixture	Radioactivity (cpm) light chain
G1 polysomes	360
G1 salt wash	80
G1 salt wash, ribosomal subunits	85
G1 polysomes, G1 salt wash	1680
G2 polysomes	120
G2 salt wash	75
G2 salt wash, ribosomal subunits	80
G2 polysomes, G2 salt wash	230
G1 polysomes, G2 salt wash	1485
G2 polysomes, G1 salt wash	395
No additions	70
High speed supernatant omitted	85
pH5 fraction omitted	75

Incubation mixtures contained : 50mM KCl, 100mM ammonium acetate, 3.5mM magnesium acetate, 6mM 2-mercaptoethanol, 10mM Tris (pH 7.4), 1.5mM ATP, 0.5mM GTP, 15mM phosphocreatine, 60μg/ml phosphocreatine kinase, approx. 0.3μ mol./ml of 20 amino acids containing 0.8μCi/ml [^{14}C]-labeled amino acids, 0.1mg/ml Krebs II ascites high speed supernatant and 1.7 A260nm units/ml of pH5 fraction from MPC-11 cells. The following additions were made to the incubation mixtures where indicated in the Table : 0.3 A260nm units of 0.5M salt washed G1 or G2 MPC-11 membrane-bound polysomes, 30μg 0.5M salt wash protein from G1 or G2 polysomes, 200μg MPC-11 ribosomal subunits. Incubation and isolation of light chain immunoglobulin was performed as indicated in Experimental.

contain polysomes or polysomes and salt wash. Light chain mRNA was apparently not extracted from polysomes by the 0.5M KCl treatment since no light chain synthesis was observed upon the addition of ribosomal subunits to 0.5M salt wash in an in vitro assay. Ribosomal subunits were isolated from MPC-11 cells according to the method of Mechler and Mach (21). Polysome

profiles of preparations treated with either 0.5M or 0.1M KCl were virtually identical indicating that there was no breakdown of the polysome structures as a result of the high salt treatment.

As expected the most effective synthesis of light chain occurred in the system where the components were from the G1 phase. The G2 system was much less effective. These in vitro observations were in fact in complete agreement with previous results where light chain synthesis was studied in intact cells (4). Rather surprisingly the G2 salt wash appeared to have almost the same stimulatory effect on G1 polysomes as those from the G1 phase, suggesting that once the mRNA was in polysomes then the set of factors present in a G2 salt wash was sufficient to enable efficient translation of the messenger to proceed. If the loss of translatory ability of light chain mRNA in the G2 phase was caused by the addition of a 'locking' factor to the informosome complex or the result of the appearance of an interference factor, similar to observations in prokaryote systems (22), then one would expect that the G2 salt wash would cause the inhibition of translation of light chain mRNA by G1 polysomes, assuming that such factors had been removed by the high salt. Inhibition, however, was not observed.

When G1 salt wash was added to G2 polysomes then stimulation above that caused by G2 salt wash was observed. Although the degree of stimulation caused by G1 salt wash on G2 polysomes was much less than that on G1 polysomes, the observation is quite reproducible. Since the G1 salt wash neither alone nor with the addition of ribosomal subunits was able to promote synthesis of light chain, the results are consistent with the suggestion that some factor present in the G1 phase is able to 'activate' the light chain mRNA present in the G2 phase and enable it to be translated. The model suggesting the production of an effector molecule is thus favoured (5).

Christman et al (23) have demonstrated that when reovirus-infected L cells are incubated in KCl-Tris medium, the viral mRNAs accumulate as 50s messenger-containing ribonucleoprotein

particles which are apparently not associated with ribosomal subunits. Upon restoration of normal growth medium the viral mRNAs were shown to rapidly enter into polysomes. Since it was possible to isolate mRNA from the 50s particles which coded for protein in vitro it was evident that the mRNA was stored as an mRNP complex during the period of incubation in KCl-Tris medium. Restoration of suitable conditions presumably resulted in immediate synthesis of the missing factor(s) enabling translation of the viral mRNAs to be resumed. A similar effect has been described by Rudland (24) who showed that in resting 3T3 fibroblasts presumptive mRNA molecules predominantly accumulated as cytoplasmic ribonucleoprotein particles, the RNA of which could be chased into polysomes by the addition of animal sera to the resting culture.

Herzberg et al (25) have shown that the loss of globin synthesising ability in mature reticulocytes is due to a loss of translatory factors and not to a loss of globin mRNA. Globin synthesis by mature ribosomes could be stimulated by the addition of salt wash from ribosomes of young cells. One can therefore postulate that control of globin synthesis in aging reticulocytes is caused by the progressive loss of translatory factors. Extrapolation of these results to the system described here would require that one of the first events occurring immediately as the cell enters G1 phase would be the synthesis of some factor(s) which would enable the mRNA stabilised in the informosome complex in the previous cell cycle, to enter polysomes and cause the onset of light chain synthesis. The translation of light chain mRNA would proceed until degradation of the factor resulted in reduced synthesis, its ultimate disappearance terminating synthesis entirely.The mRNA, in its informosomal complex, now returning to the stable form for the duration of most of S phase, the G2 phase and mitosis.

The present results substantiate the accumulating evidence that control of translation occurs at the level of mRNA discriminating factors (10-14). Furthermore, the regulation of translation of specific mRNAs during the various phases of the cell cycle may also be controlled by such factors.

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