FUNCTIONAL INTERACTION OF FREE POLYRIBOSOMES WITH THE MEMBRANE OF THE ENDOPLASMIC RETICULUM IN A CELL-FREE PROTEIN-SYNTHESIZING SYSTEM FROM PLASMACYTOMA X5563.

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Summary: In cell-free protein synthesis by the murine plasmacytoma X5563, which had become a nonproducing mutant, mixed systems with free polyribosomes and microsomes incorporated ^{14}C-amino-acid into protein 3-8 times greater than the sum of the incorporations in the individual system irrespective of S-100 concentrations. This enhancement was inhibited by lecithinase A and was markedly reduced at high KCl concentrations. Smooth endoplasmic membranes had more stimulatory activity than rough endoplasmic membranes. The results indicate that the membrane of the endoplasmic reticulum and free polyribosomes interact in the cell-free protein-synthesizing system, resulting in the enhancement of protein synthesis.

In mammalian cells there are two different kinds of polyribosomes; i.e. membrane-bound polyribosomes and free polyribosomes(1). There is evidence to indicate that the former synthesizes exportable proteins and the latter nonexportable proteins(2-5). While other differences between the two classes of polyribosomes have been studied in several aspects(6-9), and studies on the interaction of polyribosomes with endoplasmic membranes have been reported(12-14,20-22), little is known of the role for the endoplasmic membrane itself in the mechanism of protein synthesis.

The murine plasmacytoma has a highly developed endoplasmic reticulum in the cytoplasm as well as plasma cells(10) and it is a good material to study the relationship between the membrane of the endoplasmic reticulum and protein synthesis. In the course of studing this problem, using the murine plasmacytoma X5563, which had lost the capacity to produce an immunoglobulin IgG(11), we found that the endoplasmic membrane stimulated ribosomal protein synthesis

through membrane-polyribosome interactions. The results are presented in this communication.

Materials and Methods: The X5563 plasmacytoma cells have been maintained as an ascitic form by intraperitoneal transplantation in (C3H x DDD)F_1 mice at intervals of 15-20 days for several years. The cells were washed twice in 1.2 M sucrose-containing buffer by centrifugation and suspended in 0.25 M sucrose in TKM(50 mM Tris;pH7.5, 25 mM KCl, 5 mM Mg acetate) containing one tenth volume of S-100 from rat liver(15). They were mildly homogenized by 10 strokes of a glass homogenizer and centrifuged for 30 min. at 15,000 x g(postmitochondrial supernatant). Free polyribosomes, microsomes and S-100 fractions were prepared from the postmitochondrial supernatant by the method of Blobel & Potter(16). S-100 fraction was further centrifuged for 4 hrs. at 105,000 x g to sediment contaminating membranes. Protein in membrane fractions was determined by the method of Lowry et al(17)

Results: Cell-free protein synthesis by free polyribosomes and microsomes, which contain both rough and smooth membranes, and by the mixed system with free polyribosomes and microsomes were examined(Table 1). The ^{14}C-amino-acid incorporation in the mixed system was observed to be 3-8 times greater than the sum of the incorporation in the free polyribosome system and the microsome system. The grade of stimulation was at the same level without S-100 in the reaction mixtures. This indicates that the soluble factors required for protein synthesis that might be included in microsomes were not the cause for the stimulation. Puromycin, which is an inhibitor of protein synthesis, reduced the incorporation in each system. The rate of protein synthesis was enhanced from the start of incubation when time-course was examined(The data is not presented) Lecithinase A inhibited the incorporation by microsomes and more strongly reduced the increased incorporation in the mixed system

Table 1. Some characteristics of cell-free systems for protein synthesis prepared from plasmacytoma cells.

Incubation medium	^{14}C-amino-acid incorporation			Stimulation
	Free Polyribosomes	Microsomes	Mixed System	
Complete	567	340	5123	5.8
-S-100	82	61	735	5.9
-ATP,GTP,PEP Pyruvate Kinase	10	19	42	-
-Ribosomes (Microsomes)	10	10	10	-
+Puromycin 2×10^{-5} M	143(75)	41(88)	286(95)	-

( ) : % inhibition.
The complete reaction mixture contained per ml : 35 mM Tris(pH 7.5) 60 mM NH_4Cl, 30 mM KCl, 0.17 M sucrose, 6 mM Mg acetate, 6 mM 2-mercaptoethanol, 4 µg polyvinylsulfate, 2 mM ATP, 0.4 mM GTP, 10 mM PEP, 30 µg pyruvate kinase, 0.1 µCi ^{14}C-leucine(342 mCi/mmole) and 50 nM other cold amino acids, 1 mg S-100, and 0.6 mg free polyribosomes or 2 mg microsomes or both in the mixed system. It was incubated for 60 min. at 37°C and hot trichloroacetic acid insoluble precipitate was collected on glass fiber filters and the radioactivity counted on liquid scintillation counter.

at a concentration as low as it did not affect the incorporation by free polyribosomes(Table 2). This implies that the membrane of the endoplasmic reticulum plays an important role in the enhancement of protein synthesis.

It has been reported that rat liver free polyribosomes can bind to conditioned rough endoplasmic membranes from rat liver(13,20,22) and hepatomacells(21) at 0-4°C and also to smooth endoplasmic membranes at 25-37°C although the proportion of binding is smaller than in the case of detergent-treated membrane-bound polyribosomes (12), and that high KCl concentrations interfere with the binding of free polyribosomes to membranes(20,22). The result of altering KCl concentrations in each system is shown in Figure 1. The effects of KCl on ^{14}C-amino-acid incorporation in each system were observed

Table 2. Effect of lecithinase A on ^{14}C-amino-acid incorporation in the cell-free system from plasmacytoma cells.

	^{14}C-amino-acid incorporation (CPM)				Stimulation
	Free Polyribosomes	Microsomes	Mixed System	Increase	
Complete	2674	418	12014	8922	3.9
+Lecithinase					
100 µg/ml	2761(0)	273(35)	8075	4962(45)	2.6
200 µg/ml	2352(12)	244(42)	5601	3005(66)	2.2

( ) : % inhibition.
The reaction mixture was the same as described in the legand of Table 1. Lecithinase A was added to the reaction mixture at zero time.

Table 3. Stimulatory capacity of rough and smooth endoplasmic membranes in the mixed system.

	^{14}C-amino-acid incorporation (CPM)	Stimulation
Free Polyribosomes	2266	
Rough Membranes	454	
Smooth Membranes	33	
Free Polyribosomes + Rough Membranes	6126	2.3
Free Polyribosomes + Smooth Membranes	11116	4.8

Smooth membranes were prepared(18) by layering the postmitochondrial supernatant over 1.3 M sucrose-containing buffer and centrifuged for 90 min. at 105,000 x g. An intermediate layer at the junction of the sucrose solution was collected and diluted with the same volume of TKM and centrifuged for 16 hrs. The pellet was suspended in 0.25 M sucrose in TKM and used as smooth membranes. Rough membranes were prepared by the same method as described in Materials and Methods from the lower dense sucrose solution and pellets after the first centrifugation.
2 mg of protein of rough or smooth membranes was added to the reaction mixture, which was the same as described in the legand of Table 1.

to be different, which indicates that interactions between free polyribosomes and microsomes occured in the mixed system, resulting in the change in the response of the mixed system to KCl. The obser-

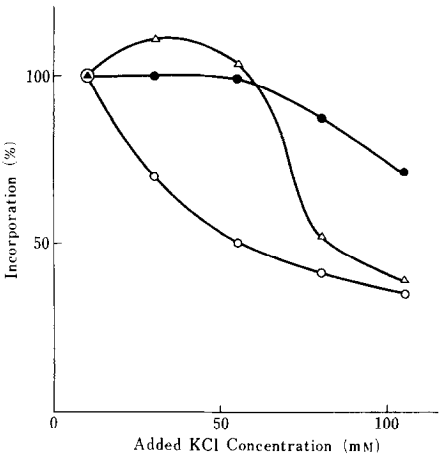

Figure 1. Effect of increasing concentration of KCl on ^{14}C-amino-acid incorporation in the cell-free system from plasmacytoma cells.

The reaction mixture was the same as described in the legand of Table 1 except KCl concentrations.
○——○ : Free Polyribosomes, ●——● : Microsomes, △——△ : Increased incorporation in the mixed system.
The absciassa is expressed as the final concentration of added KCl. The exact concentration of K^+ ion in the reaction mixture was not known, because ATP, GTP and PEP were neutralized to pH 7 with KOH.
100% was taken as the incorporation at 5 mM KCl in each system.

vation that added 80-100 mM KCl exhibited a strong inhibitory effect on the increased incorporation in the mixed system seems to be explained by the inhibitory effect of high KCl concentrations on the binding of polyribosomes to membranes.

Smooth and rough endoplasmic membranes were prepared from the plasmacytoma cells in order to investigate which related to the stimulation(Table 3). The smooth membranes did not have protein-synthesizing activity, but when free polyribosomes were combined with the same protein amount of smooth or rough membranes respectively, the smooth membranes had more stimulatory activity than the rough membranes.

Discussion: The present results indicate that the membrane of the

endoplasmic reticulum has the capacity to stimulate protein synthesis through the interaction with free polyribosomes in the cell-free protein-synthesizing system. Since membrane-bound polyribosomes can synthesize protein *in vitro* after they are treated with detergent and separated from the membrane(2), a possible role for the membrane may be an enhancement of efficiency of protein-synthesizing mechanism through membrane-polyribosome interactions. Recently it has been reported that Arrhenius activation energies of the rough endoplasmic reticulum in protein synthesis above 22°C are less than those of free ribosomes and alternations in the membrane characteristics may affect the rate of protein synthesis (19). The observation seems to be compatible with the above assumption. However, it remains another possibility that in the population of free polyribosomes there may exist membrane-dependent polyribosomes that require membranes to complete their protein synthesis.

Table 3 shows that the smooth membranes have more stimulatory activity than the rough membranes. It is conceivable that smooth membranes may have more binding sites for free polyribosomes than rough membranes, the binding sites of which are presumably occupied by loose and/or tight(23,24) membrane-bound polyribosomes(22), and thus smooth membranes may have more stimulatory activity.

Since the plasmacytoma cells used in these studies have lost the capacity to produce an immunoglobulin IgG, it can be said that this phenomenon is not specific for exportable protein synthesis, but it is not clear at present if it is also involved in the synthesis of exportable protein, which has been reported to be preferentially elaborated by tight membrane-bound polyribosomes(24). The detailed mechanism and significance in the intact neoplastic cells of this stimulatory capacity of the membrane are now under investigation.

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