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Synthesis of alpha-fetoprotein by membrane-bound polysomes of rat ascites hepatoma

Koichi KANAI, Yasuo ENDO, Toshitsugu ODA and Naomi TANAKA

The First Department of Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan

SUMMARY

Free and membrane-bound polysomes prepared from rat ascites hepatoma cells (AH–66), incorporated ^{14}C-leucine into α-fetoprotein (AFP) *in vitro*. The incorporation rate by membrane-bound polysomes was 20 to 90 times higher than that by free polysomes, indicating that AFP is mainly synthesized on membrane-bound polysomes in rat ascites hepatoma cells.

INTRODUCTION

Since Abelev[1] reported its appearance in sera of hepatoma bearing mice, detection of AFP has become an important aid for the diagnosis of primary liver cancer[2~5]. Although there are several reports concerning the production of AFP in hepatoma cells[1,6,7], little is known about the precise mode of AFP synthesis or its regulatory mechanism, or about the biological function of AFP.

There are two types of polysomes, namely free and membrane-bound polysomes in mammalian cells. Although the functional differences between these two polysomes are not completely elucidated as yet, increasing evidence suggests that free polysomes mainly synthesize proteins which are utilized within the cells and membrane-bound polysomes, on the other hand, synthesize proteins for export[8,9].

In the present study, AFP-synthesizing ability of free and membrane-bound polysomes of rat ascites hepatoma (AH–66) was investigated in an attempt to determine the intracellular site of AFP synthesis and to obtain some information as to its biological function.

MATERIALS AND METHODS

AH–66 strain of rat ascites hepatoma cells which produce AFP was transplanted intraperitoneally into male Donryu rats weighing 100 to 150 g.

Ascites cells were collected by peritoneal puncture seven days after inoculation of the tumor cells. Contaminated erythrocytes were removed by hemolysis. Less than 5% of the cells were stainable by trypan-blue after hemolysis and the tumor cells were about 98% of the total cell population.

Tumor cells were suspended in 5 ml of Krebs-Ringer bicarbonate buffer pH 7.4 (3×10^7 cells/ml) and incubated at 37°C with 5 μCi of ^{14}C-leucine for a given time. After incubation, the medium and the cells were separated by centrifugation and the proteins were analyzed for radioactivity.

Total proteins were precipitated by 10% TCA, AFP and albumin by specific antisera, respectively. Precipitated proteins were washed and then dissolved with formic acid. Radioactivity was measured on a liquid scintillation counter after adding 10 ml of scintillation fluid to the samples.

Free and membrane-bound polysomes were prepared from the tumor cells as follows: 10 to 15 g of packed tumor cells were homogenized with three volumes of the medium containing 50 mM Tris-HCl (pH 7.6), 50 mM KCl, 5 mM $MgCl_2$ and 5 mM mercaptoethanol in 0.25 M sucrose (medium A). After centrifugation at $10,000 \times g$ for 15 minutes, 2 ml of the post-mitochondrial supernatant was layered over a discontinuous sucrose gradient with 1.5 ml each of 2 M and 0.5 M sucrose in medium A. Centrifugation was performed in a Hitachi Model 55 centrifuge at $160,000 \times g$ for 3.5 hours at 4°C. Free polysomes were precipitated at the bottom of the tubes and membrane-bound polysomes were sedimented as a turbid zone between the middle and lower phases. The fraction containing bound polysomes was collected and 5% sodium deoxycholate (DOC) was added to the fraction to a final concentration of 1.3%. Subsequent centrifugation was performed at $160,000 \times g$ for 60 minutes to precipitate bound polysomes. Free polysomes were used, without further purification. A_{260}/A_{280} ratio of these two polysome-preparations ranged between 1.75 and 1.82. The supernatant fraction was prepared from rat liver according to the method of Kashiwagi *et al.*[10].

Free and bound polysomes were tested for their protein synthesizing ability in a cell-free system containing 1 mM ATP, 0.25 mM GTP, 10 mM phosphoenolpyruvate, 100 μg of pyruvate kinase, 6 mg of supernatant proteins, 2.5 μCi of ^{14}C-leucine and 1 to 2 mg of polysomes in medium A. The total volume of the reaction mixture was 2 ml. Incubation was carried out at 37°C for the period as indicated. In some experiments, EDTA was added to the reaction mixture after incubation and the incubation was continued for an additional 10 minutes. The reaction mixture was then centrifuged at $160,000 \times g$ for 60 minutes and the radioactivity of the supernatant was counted.

RESULTS AND DISCUSSION

Kinetics of the incorporation of ^{14}C-leucine into proteins of the tumor cells was shown in Fig. 1. ^{14}C-leucine was incorporated into the total proteins, AFP and also in the albumin fractions of the tumor cells. The

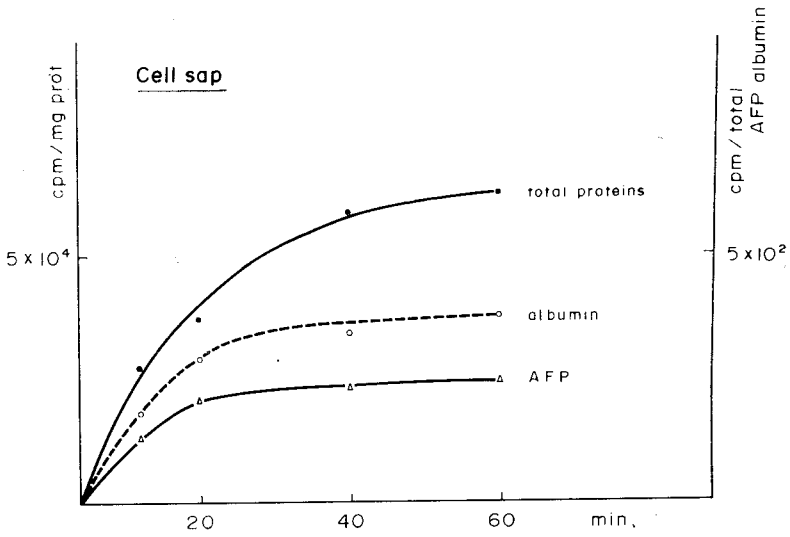

Fig. 1. Incorporation of ^{14}C-leucine into proteins of rat ascites hepatoma cells

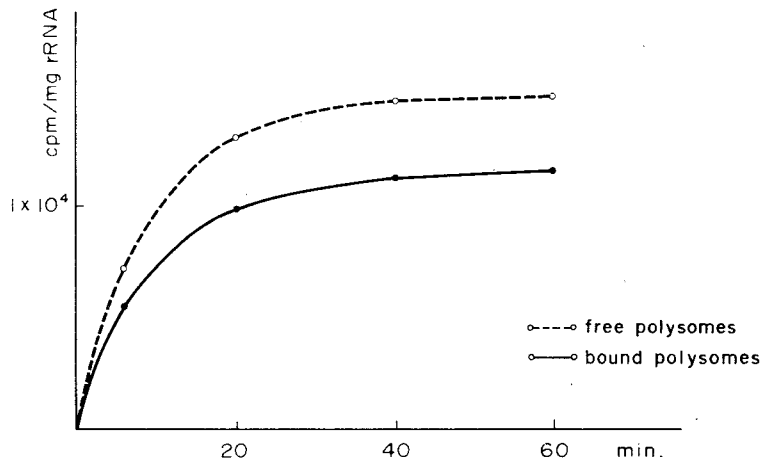

Fig. 2. Incorporation of ^{14}C-leucine into proteins by free and membrane-bound polysomes of rat ascites hepatoma cells

appearance of ^{14}C-labeled AFP and albumin in the incubation medium, therefore, seems to indicate that both proteins were synthesized by the tumor cells and subsequently secreted into the medium.

The time course of the incorporation of ^{14}C-leucine into total proteins by free and membrane-bound polysomes was presented in Fig. 2. Radioactivity increased rapidly for the first 20 minutes and reached a plateau at about 40 minutes in both free and bound polysomes. It was observed also that the activity of free polysomes which were prepared without DOC-treatment was apparently higher than that of bound polysomes treated with DOC, in agreement with the results of other investigators. After 60 minutes of incubation, 26% of the labeled materials were detected in the soluble fraction. When EDTA was added, an additional 14% of the radioactivity was released into the medium.

Synthesis of AFP by hepatoma cells has been reported, and the presence of AFP in hepatoma cells has also been confirmed morphologically. The intracellular site of AFP synthesis, however, has not been elucidated yet.

Table 1. *Incorporation of ^{14}C-leucine into AFP fraction by free and membrane-bound polysomes of rat ascites hepatoma cells*

Exp.	polysomes	radioactivity (cpm/mg RNA)		
		total proteins (T.P.)	AFP	AFP/T.P. (%)
I	free	11452	3	0.03
	bound	4384	122	2.77
II	free	12571	10	0.08
	bound	9825	152	1.54
III	free	15898	7	0.04
	bound	7813	101	1.29

As shown in Table 1, incorporation of ^{14}C-leucine into AFP fraction by membrane-bound polysomes was about 20 to 90 times higher than that by free polysomes. These results indicate clearly that AFP is synthesized mainly on bound polysomes in rat ascites hepatoma cells, suggesting that AFP belongs to "excretory protein category" such as serum albumin.

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