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Nuclear GTP-binding proteins of Swiss 3T3 cells

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The GTP-binding proteins of Swiss 3T3 cell nuclei were analyzed by filter binding assay and UV cross-linking analysis. The results showed the presence of multiple GTP-binding proteins in the nuclei. Scatchard analysis revealed that the K_d value for GTP binding to high-affinity components was 69 nM, that to low-affinity components being 2.7 μM. The GTP-binding activities of some nuclear proteins were found to change significantly in response to the growth conditions of the cells. During culture of cells in medium without serum, the GTP-binding activity of a 140 kDa protein clearly decreased, whereas that of a 40 kDa protein increased.

GTP-binding protein; Nuclear protein; Ultraviolet crosslinking; Serum starvation

1. INTRODUCTION

GTP-binding proteins in cytoplasmic membranes are known to participate in signal transduction from outside to the inside of eukaryotic cells [1–8]. These signals are finally transmitted to the nuclei in some way, resulting in induction of a set of biochemical reactions including DNA replication, however little is known about the molecular mechanism of this transmission.

Recently, GTP-binding proteins were detected in the endoplasmic reticulum and Golgi apparatus, and were supposed to be responsible for secretion of proteins [9,10]. Various GTP-binding proteins may be located in cellular organelles and participate in intracellular signal transduction. This paper describes studies on GTP-binding proteins in the nuclear fraction of Swiss 3T3 cells. Multiple GTP-binding proteins were found in the nuclei and the GTP-binding activities of some clearly changed according to the growth conditions of the cells, suggesting that these proteins are responsible for transmission of environmental signals into the nuclei.

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2. MATERIALS AND METHODS

2.1. *Cells*

Swiss 3T3 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum under 5% CO_2 in air with a relative humidity of 100% at 37°C. For culture under serum-free conditions, fetal calf serum was omitted from the medium. After cultivation for 40 h in the latter medium, the level of DNA synthesis of cells, measured as incorporation of [^{3}H]thymidine into the acid-insoluble fraction, decreased to less than 1% of the control level.

2.2. *Preparation of nuclear fraction*

The nuclear fraction was prepared as described by Franze-Fernández and Pogo [11]. Swollen cells in hypotonic buffer were homogenized with 0.3% Triton X-100. The crude nuclei obtained were washed successively with 10 mM Tris-HCl buffer (pH 7.9), containing 0.3 M sucrose, 4 mM $MgCl_2$, and 0.1 M EDTA, and the same buffer without EDTA. Washed nuclei were suspended in the former buffer and used for GTP-binding assay. This nuclear preparation was almost pure as judged by microscopic examination after staining with azure C, showing no appreciable contamination with intact cells or cell fragments.

2.3. *GTP-binding assay*

This assay was performed essentially as in [12]. The amounts of [α-^{32}P]GTP bound to nuclei were measured by trapping the nuclei onto a nitrocellulose membrane filter. The standard reaction mixture (50 μl) consisted of 10 mM Tris-HCl buffer (pH 7.9), 0.3 M sucrose, 4 mM $MgCl_2$, 1 μM [α-^{32}P]GTP (600–10000 cpm/pmol) and the nuclear fraction (50 μg protein). The reaction mixture was kept on ice for 15 min, and then filtered through a nitrocellulose membrane filter (Millipore HA

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0.45 μm). The filter was washed thoroughly with 10 ml of 50 mM Tris-HCl buffer (pH 7.9), containing 10 mM $MgCl_2$, 0.1 mM EDTA, 100 mM KCl, 5 mM β-mercaptoethanol and 20% (v/v) glycerol, dried under an infrared lamp, and its radioactivity was subsequently measured.

2.4. *UV cross-linking*

Nuclei were incubated with 1 μM [α-^{32}P]GTP under the conditions described above and then irradiated with a germicidal UV lamp for 5 min as described [13]. Materials were washed with ice-cold 10% trichloroacetic acid, subjected to SDS-polyacrylamide gel electrophoresis [14], and GTP-binding proteins were identified by autoradiography.

3. RESULTS

3.1. *GTP-binding activity of Swiss 3T3 cell nuclei*

Nuclei were prepared from Swiss 3T3 cells cultured under standard growth conditions, and their GTP-binding activity was examined. The amount of GTP trapped on a nitrocellulose membrane filter increased with increasing amounts of nuclear fraction added to the reaction mixture, reaching a saturation level of about 0.8 pmol. About 90% of the radioactivity bound to nuclei was removed when the filters were washed with 0.5 ml of 1 M formic acid (not shown), indicating that GTP binding was not due to covalent modification of nuclear proteins. Scatchard analysis [15] of the binding of GTP to a fixed amount of nuclei indicated the presence of two components of nuclear proteins with high affinity (K_d = 69 nM) and low affinity (K_d = 2.7 μM) to GTP, as shown in fig.1.

The specificity of GTP binding to the nuclei was investigated by testing the competitive effects of various nucleotides. As shown in table 1, GTP and GDP were stronger inhibitors than other nucleotides, suggesting that nuclear GTP-binding proteins have specific affinity to these two nucleotides. GMP had almost no effect on the binding of GTP. These characteristics of nuclear GTP-binding proteins are very similar to those of G-proteins in cytoplasmic membranes.

3.2. *GTP-binding activity of nuclei from cells cultured in serum-free medium*

As the above findings showed that GTP-binding proteins were present in nuclei of Swiss 3T3 cells, we subsequently investigated whether the characteristics of these proteins altered with change in growth conditions of the cells. DNA syn-

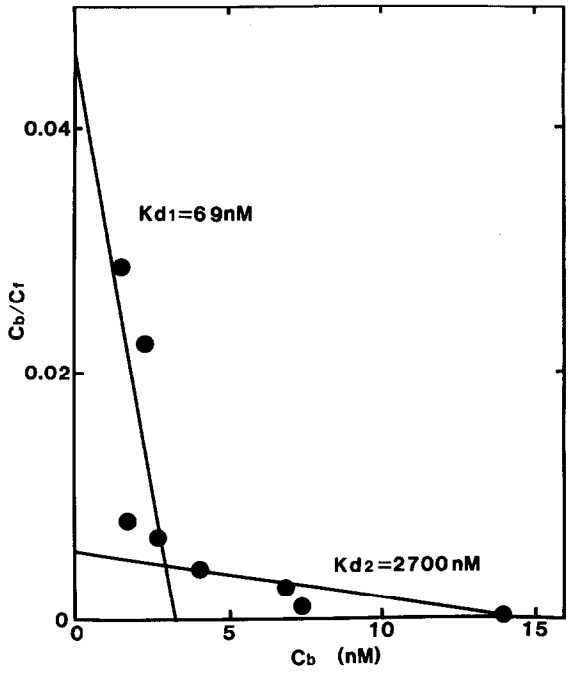

Fig.1. Scatchard analysis of GTP binding to nuclei. Nuclei were prepared from Swiss 3T3 cells cultured in normal medium for growth. GTP binding to nuclei was investigated by adding increasing amounts of [α-^{32}P]GTP to a fixed amount of nuclear fraction (50 μg protein). C_b, concentration of bound GTP; C_f, concentration of free GTP. K_d values for the low-affinity and high-affinity components are shown.

Table 1

Specificity of GTP binding to nuclei

Competitor nucleotide	Concentrations (μM) for 50% inhibition
GTP	1.2
GDP	2.8
ATP	7.5
CTP	10.0
UTP	11.2
GMP	>20

The nuclear fraction (50 μg protein) from dividing Swiss 3T3 cells was incubated with 1 μM [α-^{32}P]GTP (9500 cpm/pmol) and various concentrations of nucleotides. The amounts of bound GTP were measured by filter binding assay. The concentrations of competitor nucleotides causing 50% inhibition of GTP binding are indicated. Without competitor, 1.1 pmol GTP was retained on the membrane filter under these conditions

thesis is known to decrease markedly when cells are cultured in medium without serum. We therefore prepared nuclei from cells cultured for 40 h in serum-free medium, and compared their GTP-binding activity with that of control nuclei. As shown in fig.2, the GTP-binding activity of nuclei from serum-starved cells was about 80% of that of control nuclei.

We further compared patterns of GTP-binding proteins of these two preparations of nuclei in a cross-linking experiment. For this, the nuclei were irradiated with UV light in the presence of [α-^{32}P]GTP, and proteins containing covalently bound GTP were analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. As is evident from fig.3, several discrete bands were detected, and the overall patterns of these bands for both nuclear preparations were almost identical. However, the intensity of the band of a 140 kDa protein in nuclei from serum-starved cells was significantly lower than that of control nuclei and a band of protein of about 40 kDa was detected only in nuclei from serum-starved cells. Assuming that the intensities of the bands reflect the GTP-binding activities of the proteins, these results suggest that the GTP-binding activities of some nuclear proteins are altered with changes in the growth conditions of the cells.

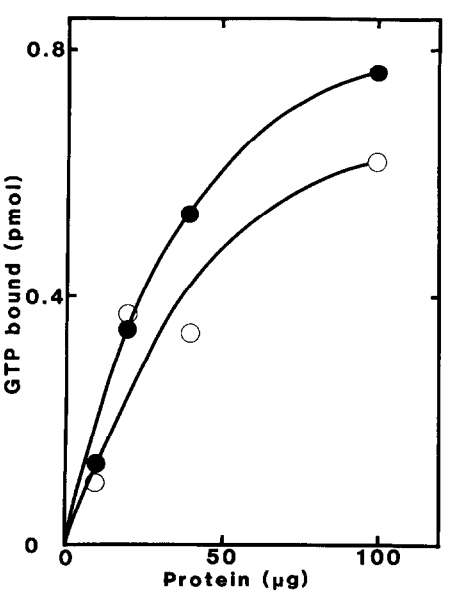

Fig.2. Binding of GTP to two nuclear fractions. Nuclei were prepared from Swiss 3T3 cells cultured in normal medium for growth and in serum-free medium. Binding of [α-^{32}P]GTP (1 μM) to increasing amounts of nuclei was measured. (●) Nuclei from normal cells, (○) nuclei from serum-starved cells.

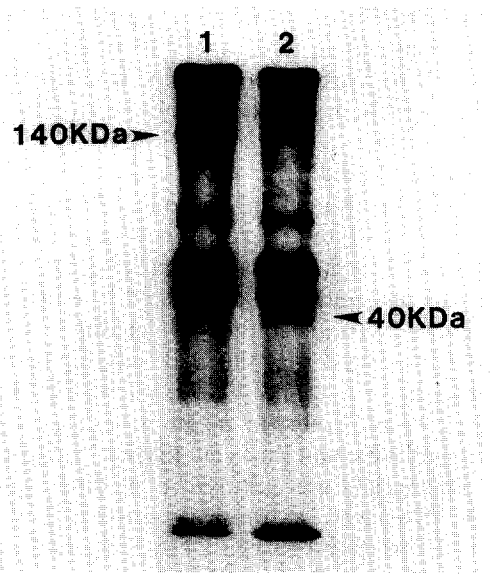

Fig.3. Analysis of GTP-binding proteins by SDS-polyacrylamide gel electrophoresis. [α-^{32}P]GTP (1 μM) was incubated with nuclei from normal or serum-starved cells. Then bound GTP was cross-linked to nuclear proteins by UV irradiation. GTP-binding proteins were analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. The gel was calibrated with the following molecular mass markers: myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa) and ovalbumin (43 kDa). Arrows indicate proteins of 140 and about 40 kDa, respectively. Lanes: 1, nuclei from normal cells; 2, nuclei from serum-starved cells.

4. DISCUSSION

We found significant GTP-binding activities in the nuclei of Swiss 3T3 cells. Nothing is known about the function of nuclear GTP-binding proteins. However, by analogy with GTP-binding proteins of the cytoplasmic membrane, it is conceivable that these proteins participate in transmission of cytoplasmic information to the nuclei. Cytoplasmic information is created by signal transduction in the cytoplasmic membrane. However, the molecular nature of the substance carrying this information is still obscure.

In general, mammalian serum is an essential component of media for in vitro culture of eukaryotic cells. Serum is known to contain

various factors that stimulate cell growth via receptors on the cell surface, and the stimuli of these growth factors are supposed to be transduced into various signals in the cytoplasmic membrane. We found that the GTP-binding activities of some nuclear GTP-binding proteins changed when cells were cultured in medium without serum. It is not certain whether the decrease in GTP-binding activity was due to a qualitative change of the protein in situ or a quantitative decrease of the protein in the nuclei during culture in medium without serum. We found that the GTP-binding activities of proteins of 140 and 40 kDa decreased and increased, respectively, in medium without serum. DNA synthesis, and thus cell division, is known to stop when cells are cultured in medium without serum. However, at present, nothing is known about the relation between DNA synthesis and the GTP-binding activities of these two proteins. Thus, further studies are required on the functions of nuclear GTP-binding proteins.

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