# UPTAKE OF RIBOSOMAL PROTEINS BY ISOLATED HELA NUCLEI

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SUMMARY

The uptake of radioactive ribosomal proteins by isolated HeLa cell nuclei has been studied. Ribosomal proteins are taken up by nuclei in vitro more rapidly than are cytosol proteins, suggesting that the uptake is selective. In addition, the ribosomal proteins are found associated with the nucleolus to a greater extent than are the cytosol proteins.

## INTRODUCTION

In eukaryote cells the communication between the nucleus and cytoplasm must be closely coordinated in order to regulate the various cellular processes. One aspect of this intracellular communication is the observation that macromolecules are transported across the nuclear membrane. Examples include the uptake by nuclei of histones and other chromatin proteins, ribosomal proteins, steroid-receptor complexes, viral DNA and enzymes for RNA and DNA synthesis. In addition, nascent ribosomes, newly assembled viruses, mRNA and tRNA are all released from the nucleus into the cytoplasm. The transport of proteins from the cytoplasm to the nucleus has been studied in several systems (for review see Refs. 1-3), although little is known about the molecular events involved in this process. In order to better understand this phenomenon and learn more about the ribosome assembly process, the uptake of ribosomal proteins into isolated HeLa nuclei has been studied using a convenient and rapid assay.

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## MATERIALS AND METHODS

All media, fetal calf serum, and media supplements were purchased from Grand Island Biological Co. [$^{14}$C]Formaldehyde (60 mCi/mmole) was obtained from New England Nuclear Corp.

Growth of HeLa Cells. HeLa S-3 cells were grown in suspension culture in Joklik modified minimum essential medium (Grand Island Biological Co.) supplemented with 5% fetal calf serum.

Isolation of Nuclei. The method used to prepare detergent-washed nuclei was essentially that of Berkowitz, et al. (4). Cells were collected by centrifugation and washed at least once in serum-free minimal essential medium. They were resuspended at a concentration of 2-4 x $10^7$ cells/ml in swelling buffer (10 mM Tris·Cl, pH 7.4; 10 mM NaCl, 1 mM $MgSO_4$) and left on ice for 14 min. The cells were then broken by 10-15 strokes with a tight pestle in a Dounce homogenizer and the nuclei pelleted at 1000 x g for 5 min. The nuclei were washed several times in a buffer containing 0.32 M sucrose, 1 mM $MgSO_4$, 5 mM Tris·Cl (pH 7.4), and either 0.3% Triton X-100 or 0.3% Nonidet P40 (NP40). It was generally found that Triton-washed nuclei were less fragile than those washed in NP40. When the nuclei were free of cytoplasmic debris, (as determined by phase contrast light microscopy), they were washed twice in a solution containing 250 mM sucrose and 1 mM $MgSO_4$ and stored in liquid nitrogen in this solution at a concentration equivalent to 1-2 mg/ml of DNA. The DNA concentration of nuclear suspensions was determined by the diphenylamine method (5) using calf thymus DNA as a standard.

For the preparation of sucrose-washed nuclei, cells were broken as described above and nuclei were pelleted and washed once in buffer containing 0.32 M sucrose, 1 mM $MgSO_4$ and 5 mM Tris·Cl (pH 7.4). The nuclei were resuspended in the same buffer and 10 ml of the suspension was layered on discontinuous sucrose gradients composed of 4 ml each of 1, 1.5 and 2 M sucrose, all containing 5 mM Tris·Cl (pH 7.4) and 1 mM $MgSO_4$. After centrifugation at 55,000 g for 75 min (Spinco SW 25.1 rotor), the pellets were resuspended in 250 mM sucrose containing 1 mM $MgSO_4$, washed twice and stored in this solution.

Preparation of Radioactive Proteins. For the preparation of radioactive ribosomal proteins, the HeLa cells were harvested by centrifugation, swollen in 2 mM $KPO_4$ buffer containing 2 mM $MgCl_2$ for 30 min at 0°C, and then homogenized by 10-15 strokes in a tight fitting Dounce homogenizer. The homogenate was sonicated 4 x 15 sec with a Bronwill Biosonik III at a maximum intensity and centrifuged for 30 min at 12,000 g to obtain a post-mitochondrial supernatant (PMS). Cytoplasmic ribosomes were pelleted from the PMS at 180,000 g for 2.5 hr and the cytosol saved. The ribosomal pellets were suspended in 0.1 M borate buffer (pH 9.0) containing 10 mM $MgCl_2$, 20 mM KCl and reductively methylated with [$^{14}$C]formaldehyde as described by Rice and Means (6). The [$^{14}$C] methylated ribosomes were then treated with 2 M LiCl at 5° for 24-48 hrs to precipitate the RNA. The precipitate was removed by centrifugation at 27,000 g for 20 min, and the supernatant containing the ribosomal proteins was removed and dialyzed extensively against 10 mM Tris-Cl (pH 7.4), 1 mM $MgCl_2$, 400 mM KCl, and 1 mM DTT.

For the preparation of radioactive cytosol proteins, the high speed supernatant containing the cytosol proteins was reductively methylated as described by Rice and Means (6). The specific activity of the ribosomal proteins was about 1000 cpm/µg and the cytosol proteins 2300 cpm/µg.

Assay for Protein Uptake. Radioactive ribosomal or cytosol proteins were incubated in a reaction mixture (100 µl) containing 50 mM Tris·Cl (pH 7.4), 5 mM $MgSO_4$, 250 mM sucrose and nuclei. The reactions were terminated

by the addition of 3 ml of a buffer containing 10 mM Tris pH 7.2, 0.32 M sucrose, 1 mM $MgCl_2$ and 0.3% Triton 101 and then filtered through a disc of Whatman #1 filter paper. The filter was washed with 3 x 10 ml of the above buffer to minimize non-specific adsorption of proteins to the nuclei, and the radioactivity determined in a liquid scintillation spectrometer using a naphthalene dioxane scintillation fluor (7).

Localization of Proteins After Uptake. Following an incubation of the nuclei with the labeled proteins, the nuclei were recovered by centrifugation through 1 ml of 15% sucrose containing 5 mM $MgSO_4$ for 5 min at 700 g. The pelleted nuclei were then washed by suspending them in 1 ml of 15% sucrose containing 5 mM $MgSO_4$, followed by centrifugation for 5 min at 700 g. The washed nuclei were suspended in 1 ml of a buffer containing 20 mM Tris·Cl (pH 7.4), 120 mM KCl, 10 mM $MgCl_2$ and 1 mM DTT, and sonicated for 4 x 15 sec with a Bronwill Biosonik III sonicator. The sonicate was layered over 1 ml of 0.88 M sucrose and centrifuged at 2000 g for 10 min. The supernatant nucleoplasmic fraction was removed and the nucleolar pellet was suspended in 1 ml of a buffer containing 10 mM Tris (pH 7.4), 10 mM NaCl and 10 mM DTT. The labeled proteins in the nucleolar and nucleoplasmic fractions were precipitated with 10% TCA, filtered through a nitrocellulose filter and assayed for radioactivity.

## RESULTS AND DISCUSSION

Uptake of Ribosomal Proteins. Since it is known that ribosomal proteins are synthesized in the cytoplasm and then transported into the nucleus where ribosomes are assembled, the uptake of ribosomal proteins could represent a useful model system for the study of transport of proteins into the nucleus. Fig. 1A shows the kinetics and the effect of temperature on the uptake of ribosomal proteins into HeLa cell nuclei. As a comparison, randomly labeled cytosol proteins were also examined (Fig. 1B). It can be seen that the uptake of ribosomal proteins at 37° is quite rapid with the reaction completed by one minute. In contrast, the uptake of cytoplasmic protein is significantly slower. Under the conditions used, from 14-21% of the ribosomal proteins were associated with the nuclei whereas only 2-7% of the cytosol proteins were taken up. In addition, the uptake of ribosomal proteins takes place just as efficiently at 0° as at 37°, whereas at 0° the cytosol proteins are taken up very poorly.

To distinguish uptake from non-specific adsorption to the nuclear envelope, the localization of the radioactive proteins inside the nucleus was examined after the incubation. It is now accepted that ribosomes are assembled in the nucleolus (8-11), and under physiological conditions ribosomal proteins would be expected to be preferentially associated with these sub-

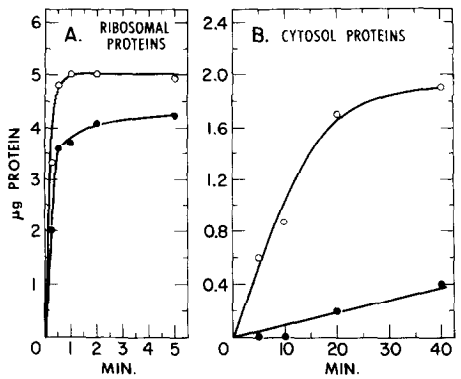


Fig. 1: Time course of the uptake of labeled ribosomal and cytosol proteins into HeLa nuclei. The incubation conditions and assay are described in the text. The incubations contained 25.2 µg of ribosomal proteins, 27.0 µg of cytosol proteins and 18.6 µg of DNA.

○ - 37°

● - 0°

TABLE I

The Intranuclear Distribution of Proteins Taken Up by HeLa Nuclei

| Protein | Uptake µg | Nucleoplasm % | Nucleolus % |
|---|---|---|---|
| Ribosomal | 34.6 | 28 | 72 |
| Cytosol | 6.7 | 63 | 37 |

The incubation conditions and assay are described in the text except that the incubations were scaled up to 1 ml and contained 270 µg S-200 and 252 µg of ribosomal proteins. After 30 min at 37° an aliquot was removed for the determination of the amount of protein taken up and the remainder was separated into a nucleolar and nucleoplasm fraction as described in the text.

nuclear particles. Table 1 shows that over 70% of the ribosomal proteins taken up by nuclei are found associated with the nucleolus, whereas only 37% of the

cytosol proteins are found in the nucleolus.

There were several other characteristics of the system worth noting. The proteins used in these studies were labeled in vitro by reductive methylation; however, similar results were obtained employing proteins that were labeled in vivo. Detergent washed nuclei (0.3% Triton) used here gave similar results as non-detergent treated nuclei suggesting that the nuclear membrane was not required for this in vitro system. Since the nuclei used were stripped of their nuclear membranes and the uptake of ribosomal proteins took place at 0°, an active uptake is probably not involved. Although this process does not appear to be energy linked, the system is of interest because of the specificity, the kinetics of uptake and intranuclear localization.

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