THE TRANSFER OF PROTEINS FROM CYTOPLASM TO NUCLEUS IN HeLa CELLS.*

Henry L. Speer** and Ernest F. Zimmerman***

Department of Pharmacology
Stanford University School of Medicine
Palo Alto, California

Received June 7, 1968

There is some question as to the origin of nuclear proteins in eukaryotic cells. Birnstiel and Flamm (1964) present evidence that in pea seedlings, histones are synthesized in the nucleus, and Frenster et al. (1961) show the incorporation of amino acids into proteins by ribosomes isolated from calf thymus nuclei. Conversely Robbins and Borun (1967) conclude that histones are synthesized in the cytoplasm of HeLa cells. Goldstein and Prescott (1967) using Amoeba proteus have divided nuclear proteins into two classes: one class that migrates rapidly back and forth between nucleus and cytoplasm and a second class that migrates more slowly. Other data (Byers et al., 1963) suggest that the proteins investigated in Amoeba proteus are synthesized in the cytoplasm.

Penman (1966) describes a detergent treatment for the preparation of HeLa nuclei which, as shown by electron microscopy, are free of contamination by cytoplasmic ribosomes (Holtzman et al., 1966). Extraction of these nuclei show only 0.2% of cellular 18S rRNA to be present, indicating that in HeLa cells the nucleus

*This research was supported by a grant (T39) from the American Cancer Society.

**Present address: Department of Biological Sciences, Simon Fraser University, Burnaby 2, B.C., Canada.

***Present address: Institute for Developmental Research, Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, Ohio 45229.

does not contain an appreciable percentage of the cells ribosomes (Penman et al., 1966). From the foregoing there would seem to be some controversy as to the existence of nuclear ribosomes and therefore the origin of nuclear proteins, at least in HeLa cells. In the cases where nuclear protein synthesis and nuclear ribosomes have been demonstrated, Holtzman's data would seem to indicate that contamination by cytoplasmic ribosomes may have been a factor. There is also the possibility that HeLa cell nuclei may differ from other cells investigated in that they do not contain nuclear ribosomes.

This paper is an investigation of the kinetics of the transfer of H^3-labeled proteins from the cytoplasm into the nucleus of HeLa cells. Detergent-treated nuclei are used to prepare both whole nuclei and nuclear fractions and the distribution of H^3-labeled proteins in the nuclear fractions is investigated. The influence of the drug cycloheximide on the migration of H^3-labeled proteins is also examined.

Results and discussion:

The incorporation of H^3-leucine into the cytoplasm and nucleus of exponentially growing HeLa cells proceeds so rapidly at 37^o that it is difficult to label the cytoplasm without a correspondingly high degree of radioactivity appearing in the nucleus. In order to overcome this difficulty, cells were incubated at 25^o and the kinetics of H^3-leucine incorporation into cytoplasmic and nuclear proteins were compared with those at 37^o (Figure 1). At 25^o, the distribution of radioactivity into each cell fraction was the same as 37^o; the rate was depressed by 90%.

Using this technique, HeLa cells were pulsed with H^3-leucine at 25^o for 1 min, which allows appreciable labeling of the cytoplasmic proteins with a minimal labeling of nuclear proteins, and then chased for various periods of time in the absence of protein synthesis. The distribution of radioactive proteins in whole cells, cytoplasm and nucleus was then followed in order to see if radioactive cytoplasmic proteins enter into the nucleus. Figure 2 shows that the incorporation of H^3-leucine

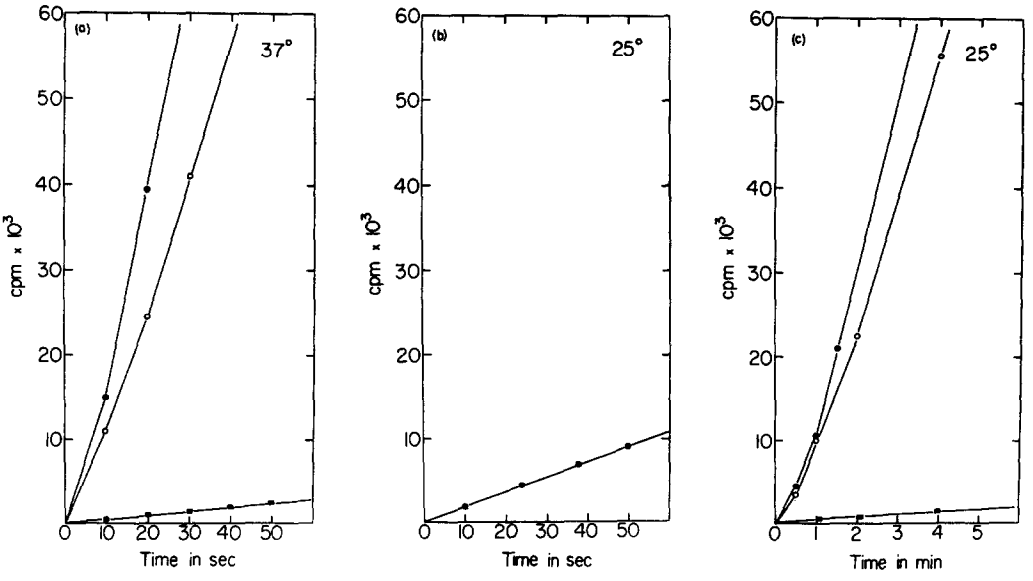
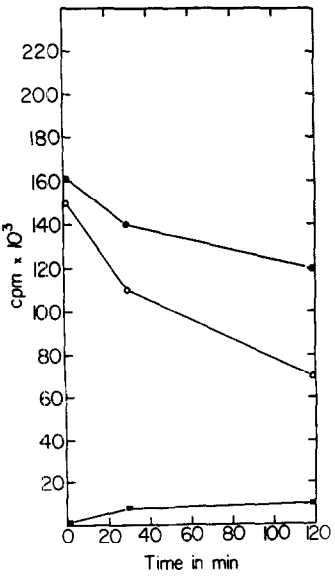


FIGURE 1. Kinetics of protein synthesis in HeLa cells and cell fractions at 37° and 25°. Exponentially growing monolayer cells (Zimmerman and Holler, 1967) were scraped and washed twice in leucine-free medium containing 10% dialyzed calf serum and resuspended in the same medium at 2 x 10^7 cells/ml. Incorporation of H^3-leucine (5000 μc/μmole, 7 μc/ml) at indicated periods of time was stopped by pouring cell suspension over crushed frozen Earle's saline. Cytoplasm and nuclei were prepared by detergent treatment (Penman, 1966). All fractions were precipitated in 5% TCA and heated to 90° for 30 min. Precipitates were collected on Whatman GF/C glass filters, washed 5 times with 5% TCA, digested in NCS (quaternary ammonium hydroxide supplied by Nuclear Chicago Corp.) overnight at 37° and radioactivity was measured in a toluene-PPO-POPOP mixture. H^3-Toluene was later added as an internal standard for quench correction. Whole cells, • ; cytoplasm, o ; nuclei, ▪ (a) cells incubated at 37°, (b) cells incubated at 25°, (c) cells incubated at 25° for several min.

---

into proteins of whole cells and cytoplasm decreases with time of chase, probably due to turnover of proteins, while the incorporation into the nucleus increases. The effectiveness of cycloheximide inhibition was next tested by addition of H^3-leucine to the cell suspension containing complete medium and cycloheximide at 37°. The incorporation of H^3-leucine into whole cells under these conditions was inhibited by 95%. Figure 3 shows that negligible radioactivity is incorporated into the nucleus



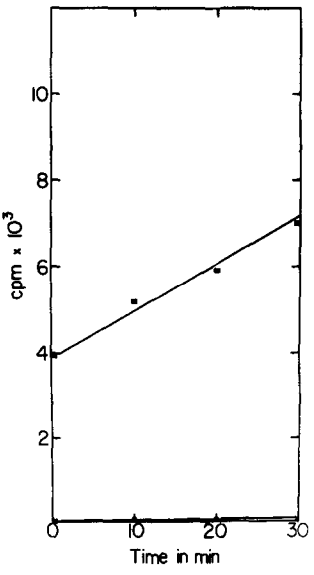


FIGURE 2. (Left) The kinetics of the transfer of proteins labeled at 25° for 1 min from the cytoplasm into the nucleus in the absence of protein synthesis. After incorporation of H^3-leucine (5000 μc/μmole) for 1 min at 25°, cells were emptied into 10 volumes of complete medium at 37° which contained 100 μg/ml of cycloheximide. Cell fractions were prepared and radioactivity measured as in Figure 1. Whole cells, ● ; cytoplasm, o ; nuclei, ▪

FIGURE 3. (Right) Effect of cycloheximide on incorporation of radioactivity into nuclei. ▪ , Cells were prelabeled with H^3-leucine (5000 μc/μmole) for 1 min at 25° and transferred to 10 volumes of complete medium containing 100 μg/ml cycloheximide at 37°. X, Cells were labeled in 10 volumes of complete medium containing 100 μg/ml cycloheximide at 37°. Nuclei were prepared and radioactivity counted as in Figure 1.

---

when cycloheximide is present at the time of addition of H^3-leucine; the labeling that does occur is restricted almost entirely to the cytoplasm.

Since there was no nuclear protein synthesis under these chase conditions, these experiments indicate that there is a transfer of radioactive proteins from the cytoplasm into the nucleus. However, since cycloheximide is inhibiting further protein synthesis and consequently the synthesis of enzymes and RNA (Ennis, 1966), a pulse-chase experiment was carried out in the absence of cycloheximide. Cells

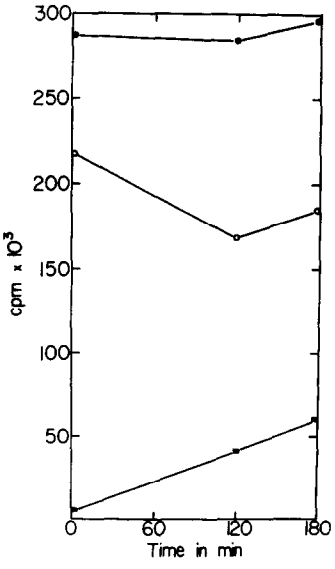


FIGURE 4. The distribution of proteins labeled for 1 min at 25° into whole cells, cytoplasm and nuclei under chase conditions where protein synthesis continues. Cells were exposed to H^3-leucine (60,000 μc/μmole, 7 μc/ml) for 1 min at 25° and transferred to 10 volumes of ice cold complete medium, washed three times in this medium and resuspended in complete medium at 37°.

were extensively washed in the cold before incubation at 37°. Figure 4 shows that the pattern of distribution of labeled proteins is quite similar to the previous experiments in which cycloheximide was used. The apparent increase in radioactivity incorporated is due to the increase of specific activity of H^3-leucine (60,000 μc/μmole) employed. This experiment was carried out for 3 hours during which time the transfer of proteins from the cytoplasm into the nucleus remained linear and represented a 9-fold increase in nuclear labeling. Therefore continued synthesis of proteins may be a factor in the transfer of proteins from cytoplasm to the nucleus, since the transfer of proteins into the nucleus leveled off at the end of about 30 min, when cells were incubated in the presence of cycloheximide (Figure 2).

Nuclei, from cells labeled for 1 min at 25° with H^3-leucine and then chased for 30, 60, and 90 min in complete medium containing cycloheximide, were lysed by the use of 0.5% DOC and 100 μg DNase in 1 ml RSB buffer (0.01 M Tris, pH 7.4; 0.01 M NaCl; 0.0015 M $MgCl_2$) and particulate material was removed by centrifugation at 25,000 g for 15 min. The nuclear supernatant was then layered over a 5-30%

sucrose gradient in RSB and centrifuged in a Spinco SW-50 rotor at 50,000 rpm for 2 hr. No radioactivity was found in the gradient where cytoplasmic ribosomal particles (78S, 60S and 40S) would appear. Fifty-seven per cent of the total radioactivity was present in the 4S region of the gradient and 43% in the pellet.

Next the distribution of radioactive proteins into nucleoli and nucleoplasm was measured, the same pulse-chase conditions being employed. It was observed that 51% of the radioactivity of the nuclear lysate (DOC-DNase-treated) was in the nucleolar pellet (30,000 g and 15 min centrifugation in RSB buffer) and 49% in the nucleoplasmic supernatant fraction. Identical results were obtained when high salt buffer (0.5 M NaCl; 0.05 M $MgCl_2$; 0.01 M Tris, pH 7.4) was substituted for RSB buffer.

In summary, these experiments indicate that there is a transfer of protein from the cytoplasm of HeLa cells into the nucleus. This transfer is linear with time. The protein transferred into the nucleus was not observed to be associated with nuclear ribosomal particles, but consists of a soluble fraction (4S) and an insoluble one found in the nucleolus. Further work is underway at present in an attempt to characterize these proteins.

REFERENCES

Birnstiel, M. L. and Flamm, W. G. (1964). Science 145, 1435.
Byers, T. J., Platt, D. B. and Goldstein, L. (1963). J. Cell Biol. 19, 467.
Ennis, H. L. (1966). Mol. Pharmacol. 2, 543.
Ennis, H. L. and Lubin, M. (1964). Science 146, 1474.
Frenster, J. H., Allfrey, V. G. and Mirsky, A. E. (1961). Biochem. Biophys. Acta 47, 130.
Goldstein, L. and Prescott, D. M. (1967). J. Cell Biol. 33, 637.
Holtzman, E., Smith, J. and Penman, S. (1966). J. Mol. Biol. 17, 131.
Penman, S. (1966). J. Mol. Biol. 17, 117.
Penman, S., Smith, I. and Holtzman, E. (1966). Science 154, 786.
Robbins, E. and Borun, T. W. (1967). Proc. Nat. Acad. Sci. 57, 409.
Zimmerman, E. F. and Holler, B. W. (1967). J. Mol. Biol. 23, 149.