CYTOPLASMIC SYNTHESIS OF ACIDIC CHROMOSOMAL PROTEINS*

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SUMMARY. The site of synthesis of acidic chromosomal proteins was studied by pulse labeling exponentially growing HeLa cells with leucine-^{3}H at 20° and determining the amount of radioactivity incorporated into proteins after chasing for various periods of time, or after addition of cycloheximide, an inhibitor of protein synthesis. The incorporation of leucine-^{3}H into total cellular, cytoplasmic, total nuclear and acidic nuclear proteins was measured. The results indicate that acidic chromosomal proteins are largely synthesized in the cytoplasm and then transferred to the nucleus; however, the possibility of some protein synthesis within the nucleus cannot be completely eliminated.

INTRODUCTION

Acidic chromosomal proteins are believed by a number of investigators to have a regulatory function on the mammalian genome and a considerable amount of attention has been directed recently towards their role in the control of gene expression in general (1-8) and cell proliferation in particular (9-12).

Robbins and Borun (13) and Gallwitz and Mueller (14) have demonstrated that HeLa cell histones are synthesized in the cytoplasm on small polysomes, but the site of synthesis of acidic chromosomal protein has not yet been reported. The purpose of the present investigation was to determine whether acidic chromosomal proteins of HeLa cells are synthesized in the nucleus or in the cytoplasm.

METHODS. Exponentially growing HeLa S_3 cells were maintained in suspension culture at 20° for 60 minutes and then pulse labeled for 2 minutes

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at a concentration of 5 x 10^6 cells/ml in leucine-free Eagle's Minimum Essential Medium (MEM) to which were added 33 μCi/ml of l-leucine-^{3}H (58 Ci/mmole, New England Nuclear Corporation). An aliquot of pulse-labeled cells (2.5 x 10^7 cells) was removed, diluted with 10 volumes of iced Earle's salt solution (EBS) and pelleted. The other cells were pelleted by centrifugation (start of the "chase"), resuspended at a concentration of 5 x 10^5 cells/ml in MEM (containing normal amount of leucine; 52 mg/l) supplemented with 3.5% each of calf and fetal calf serum and maintained at 20°. At 5, 15, 30 and 60 minutes after the start of the chase, samples consisting of 2.5 x 10^7 cells were removed and pelleted. All samples were washed 3 times with 40 volumes of EBS and the nuclei were isolated by 3 washings in 80 mM NaCl, 20 mM EDTA and 1% Triton X-100 (15). The nuclei were then extracted twice with 0.15 M NaCl, twice with 0.35 M NaCl (salt-soluble acidic proteins) and a crude histone extract was obtained by 3 washings with 0.25 N H_2SO_4. The pellet was extracted with hot 5% trichloracetic acid to remove nucleic acids, and the amount of DNA was determined by Burton's modification of the diphenylamine reaction (16). The remaining pellet, containing the acidic chromosomal proteins, was solubilized in 1 N NaOH. The incorporation of leucine-^{3}H into total cellular, total nuclear and cytoplasmic proteins was determined by precipitation with 1 N perchloric acid and solubilization of the precipitates in 1 N NaOH. 0.1 ml aliquots of each sample were added to 10 ml of Liquifluor-Toluene (New England Nuclear Corporation) and counted in a Packard Tri-Carb Liquid Scintillation Spectrometer. 0.1 ml aliquots of each nuclear protein fraction were counted in a similar manner.

RESULTS. Figure 1a shows that under these conditions there are no significant fluctuations in the amount of leucine-^{3}H in total cellular protein between the end of the pulse and the end of the chase, 60 minutes later. However, there is an apparent loss of radioactivity from cytoplasmic proteins with a corresponding increase in the nuclear protein. Figure 1b

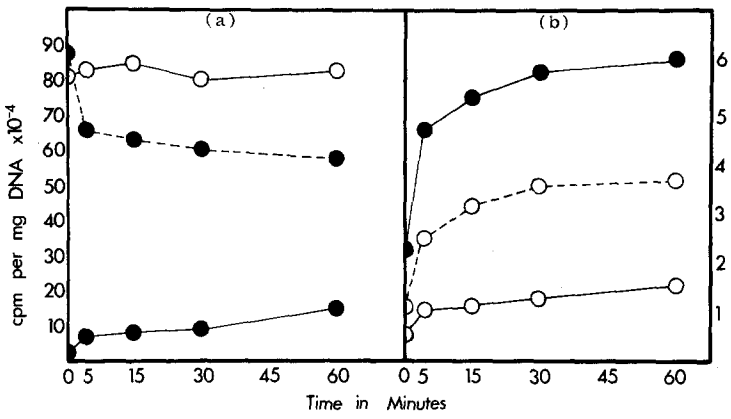

Fig. 1 (a) Incorporation of 1-leucine-^{3}H into total cellular (○—○—○), nuclear (●—●—●) and cytoplasmic (●---●---●) proteins at various times following a 2-minute pulse with leucine-^{3}H.

(b) Incorporation of 1-leucine-^{3}H into 3 nuclear protein fractions at various times following a 2-minute pulse. 0.35 M NaCl fraction (○—○), histones (○---○), acidic chromosomal proteins (●—●).
The ordinate gives the amount of radioactivity incorporated into proteins per mg of DNA; the abscissa gives time in minutes after the end of the pulse.

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shows that the amount of radioactivity in histones as well as in two acidic chromosomal protein fractions (12) increases between the pulse and the end of the chase. These results suggest that nuclear proteins synthesized in the cytoplasm in the first 2 minutes of the experiment are entering the nucleus in the subsequent 60 minutes. Furthermore, since the cytoplasmic synthesis of HeLa cell histones has been clearly established (13), and since the kinetics of transfer from the cytoplasm to the nucleus are similar in acidic chromosomal proteins and histones, it appears that acidic chromosomal proteins are synthesized in the cytoplasm.

In a second experiment, in order to further minimize the labeling of nuclear proteins during the pulse and to eliminate the possibility of isotope reutilization, cells were pulsed as previously described with leucine-^{3}H for 30 seconds at 20° and then transferred to 10 volumes of 37° MEM, with the normal amount of leucine, containing 3.5% each of calf and fetal

calf serum and 5 µg/ml of cycloheximide. At this concentration cycloheximide inhibits protein synthesis by more than 95%. Figure 2a shows that there is a loss of radioactivity from cytoplasmic proteins between the end of the pulse and the end of the incubation in the presence of cycloheximide 60 minutes later, with a corresponding increase in labeled nuclear protein, again suggesting a transfer of radioactive proteins from the cytoplasm to the nucleus. The increase in radioactivity of histone and acidic chromosomal proteins in the absence of protein synthesis for 60 minutes after a 30-second pulse (Fig. 2b) is a further indication that these proteins are synthesized in the cytoplasm.

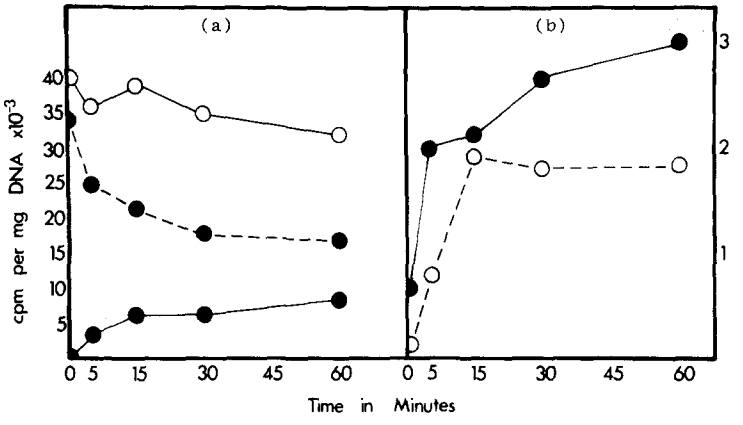

Fig. 2 (a) Incorporation of l-leucine-^{3}H into total cellular (○—○), nuclear (●—●) and cytoplasmic (●---●) proteins at various times after a 30-second pulse followed by the addition of 5 µg/ml of cycloheximide.

(b) Incorporation of l-leucine-^{3}H into histones (○---○) and acidic chromosomal proteins (●—●) under conditions described in 2 (a). Ordinate and abscissa as in Fig. 1.

DISCUSSION. These results clearly suggest that acidic chromosomal proteins are synthesized in the cytoplasm and then are transferred into the nucleus, which is consistent with the observation of Speer and Zimmerman (17) on the transfer of newly synthesized proteins from the cytoplasm to the nucleus of HeLa cells; yet, they do not eliminate the possibility that some nuclear proteins may also be synthesized within the nucleus. It

should be pointed out that even after a 30-second pulse at 20° some acid-precipitable radioactivity is present in nuclear proteins. Under these conditions, 8.5% of the radioactivity found in nuclear proteins after a 60-minute chase is already in the nucleus at the termination of a 30-second pulse. It has been claimed that isolated nuclei from a variety of plant and animal tissues are capable of incorporating amino acids into proteins *in vitro* (18-23). In fact, Frenster *et al.* (24-25) showed that ribosomes isolated from calf thymus nuclei are capable of supporting amino acid incorporation into proteins, and although the HeLa cell nucleus does not contain a significant percentage of cell ribosomes, Gallwitz and Mueller (14) have shown that HeLa cell nuclei are capable of synthesizing proteins *in vitro* which differ from those synthesized on the microsomes of the same cells under similar conditions. Even if some nuclear protein synthesis does occur, our results indicate that it amounts to less than 10% of the newly synthesized proteins that are found in the nucleus one hour after a 30-second pulse. We can, therefore, conclude that more than 90% of the nuclear proteins (including acidic chromosomal proteins) are synthesized in the cytoplasm and then transferred to the nucleus.

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