POLYSOMAL SITE OF PROTEIN SYNTHESIS IN HELA CELLS*

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Investigations on protein synthesis in bacteria have elucidated the following scheme: genetic information from the DNA genome is transferred to the newly synthesized protein by mediation of a rapidly metabolizing RNA molecule (messenger RNA) which after attachment to ribosomal particles determines the amino acid sequence of the protein (Jacob and Monod, 1961; Gros et al., 1961; Brenner et al., 1961). Experiments which show that the messenger RNA is attached to the ribosome are well documented. Risebrough et al. (1962) labeled T2 phage messenger RNA and observed formation of "heavy ribosomes" from 70S ribosomes of E. coli by sucrose sedimentation analysis. Complexes of ribosomes are seen when synthetic polyuridylic acid is mixed with cell-free extracts of bacteria (Spyrides and Lipmann, 1962; Barondes and Nirenberg, 1962). Warner et al. (1962, 1963) have shown that in reticulocytes, animal cells which do not synthesize RNA, protein synthesis occurs on ribosome clusters (polysomes). In the HeLa cell, I have recently observed a similar synthesis of proteins on heavy particulate matter, the nature of which will be discussed.

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Exponentially growing HeLa cells with a generation time of 18-24 hours were used as the source of biologic material (Eagle, 1959; Zimmerman et al., 1963). Cells were washed with warmed leucine-free medium, resuspended in the same medium at a concentration of 4 x 10^6 cells/ml and incubated for 30 minutes at 37° to exhaust the endogenous leucine pool, after which time the isotopically labeled amino acid was added. In order to be certain that this manipulation in a leucine-free medium did not impair protein synthesis, the rate of protein synthesis was followed at times beginning at 15 seconds after the "wash-out" period. As Fig. 1 indicates, the initial uptake of leucine-H^3 into HeLa protein was linear. The curves did not extrapolate to zero, perhaps because of some non-specific binding of the isotope.

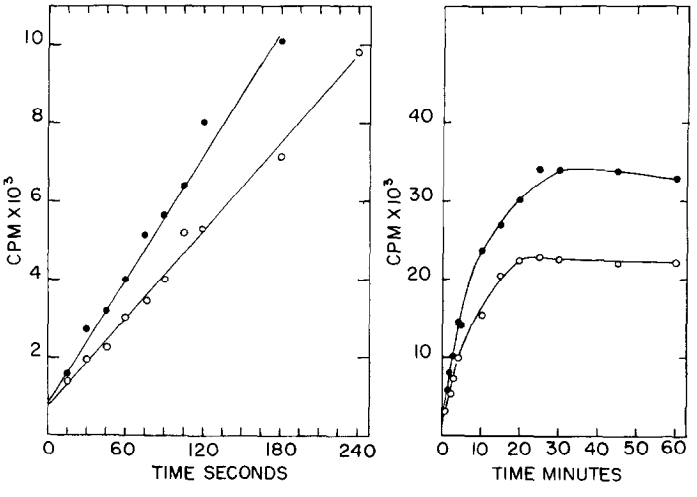

Fig. 1. Kinetics of protein synthesis in HeLa cells. Open Circles: medium with 5% horse serum. Closed Circles: medium lacking horse serum. Same experiment plotted with second and minute scales. 1.6 x 10^7 cells washed and resuspended with 4 ml of suitable medium lacking leucine. Cell suspension incubated for 30 minutes at 37° at which time dl-leucine-4,5-H^3 (5.45 c/mmole, 100 μc) was added to the suspension. At suitable times, 0.1 ml aliquots were pipetted into 2 ml of 5% trichloracetic acid, precipitates collected on Millipore filters, and radioactivity counted in a Packard Scintillation Counter.

Protein synthesis apparently was proceeding at a maximal rate since the cells used up the limiting quantity of leucine in 20-25 minutes, as compared with the calculated time of 22.5 minutes assuming exponential growth with a generation time of 24 hours. During the time period studied, the presence of serum in the medium did not enhance incorporation. In fact, protein synthesis was depressed by serum which might be due to serum protein binding of leucine. Serum was therefore omitted in all subsequent experiments.

To determine whether proteins were synthesized on single ribosomes or on clusters, cells treated as previously described were exposed for 60 seconds to tritiated leucine. Upon sucrose gradient analysis (15-30%) it is seen in Fig. 2a that radioactivity is scattered throughout the gradient. If the 10,000 x g supernatant was first treated with 0.5% sodium desoxycholate, a lipid solvent known to dissolve membranes (Palade and Siekevitz, 1956), there appeared in the gradient (Fig. 2b) a broad peak of radioactivity, the center of which was calculated to be about 253S (Nomura, Hall and Spiegelman, 1960). Puromycin (10^{-4} M) could be shown to prevent the appearance of this broad band of radioactivity associated with the heavy cell material (Fig. 3).

If proteins are synthesized on clusters of ribosome particles held together by messenger RNA molecules, then a mild treatment with RNAase might hydrolyze these unstable messenger RNA's while not extensively hydrolyzing the ribosomal RNA material. And if proteins are synthesized on the ribosomal particle, then such a treatment should transfer the radioactivity of the newly synthesized proteins from a broad-banded, heavy fraction to the lighter 78S ribosome (Risebrough et al., 1962; Warner et al., 1962, 1963). In

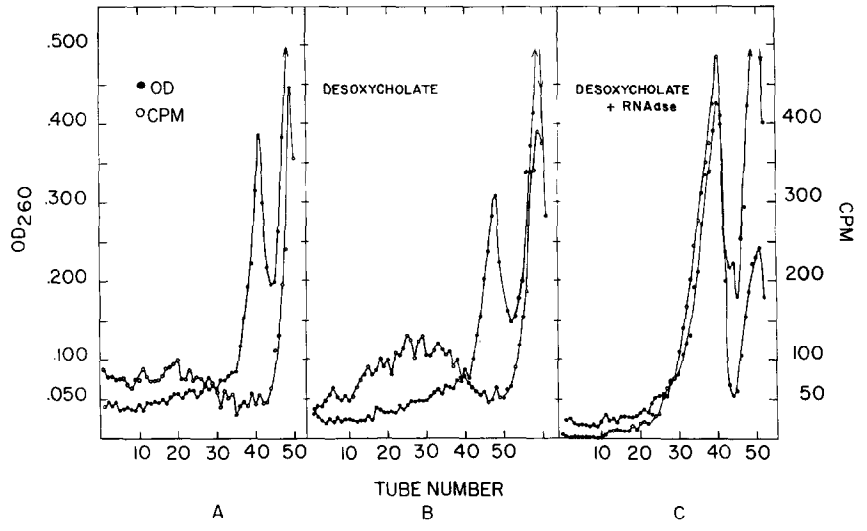

Fig. 2. Sucrose gradient analysis of protein synthesis from HeLa cells. 1.8 x 10^8 cells were washed, preincubated in leucine-free medium for 30 minutes at 37° and exposed for 60 seconds to 100 μc of dl-leucine-4,5-H^3 (5.45 c/mmole). Incorporation of isotope was stopped by pouring cell suspension into twice the volume of crushed ice medium. Cells harvested by centrifugation, resuspended in 6 ml distilled water, incubated at 5° for 20 minutes, homogenized with 10 strokes using a loose fitting pestle in a Dounce homogenizer. Concentrated buffer added to yield 0.05 M Tris (pH 7.6), 0.025 M KCl, 0.0025 M $MgCl_2$ and 0.25 M sucrose. Homogenate centrifuged at 10,000 x g for 15 minutes. 1.5 ml of supernatant added to 25 ml of a linear gradient of sucrose (15-30% W/W) containing the standard buffer. Samples spun for 2 hours, at 25,000 rpm in a SW 25.1 Spinco rotor at 5°. A, control; B, sodium desoxycholate (0.5%) treatment of 10,000 x g supernatant for 15 minutes at 5°, then put on gradient; C, ribonuclease (0.25 μg/ml) treatment of 10,000 x g supernatant at 5° for one hour, followed by sodium desoxycholate treatment.

order to test this, the 10,000 x g supernatant from labeled HeLa cells was treated with 0.25 μg/ml of RNAase for 60 minutes at 5°, treated with sodium desoxycholate and then analyzed by sucrose gradient analysis. The results are shown in Fig. 2c. It can be observed that the radioactivity has been shifted over to the 78S ribosome.

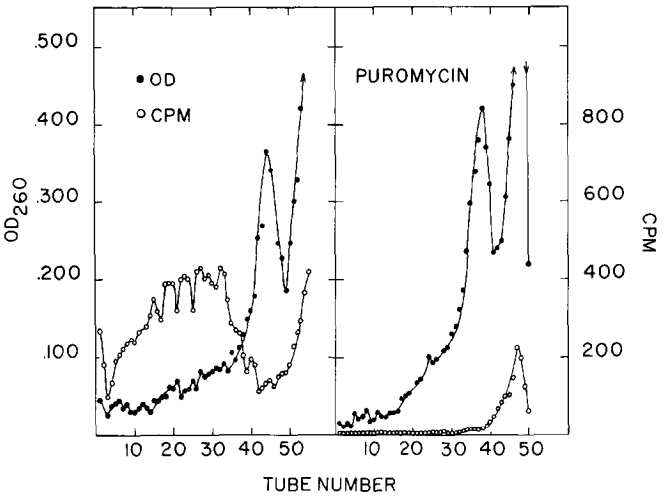

Fig. 3. Effect of puromycin on protein synthesis. Conditions same as Fig. 2 except that one sample was preincubated with 10^{-4} M puromycin for 30 minutes before leucine-H^3 was added. Sodium desoxycholate treatment included.

In summary, the experiments show that labeled leucine is incorporated into a fraction, which on sucrose gradient analysis, and after treatment with sodium desoxycholate, appears as a broad band with an average sedimentation coefficient of 253S. The incorporation can be blocked with puromycin and its distribution in the gradient can be shifted by mild RNAase treatment to a lighter peak which is indistinguishable from the 78S ribosomal peak. These data are consistent with the hypothesis that proteins are synthesized in HeLa on polysomes, clusters of ribosomes attached by labile RNA molecules. It is probable that these labile RNA molecules should serve the messenger function, since polyuridylic acid is known to polymerize ribosomes in bacterial extracts (Spyrides and Lipmann, 1962; Barondes and Nirenberg, 1962). The observation that desoxycholate affords sharper delineation of the peak (Fig. 2b) suggests that the polysomes themselves are attached to lipid membranes, such

as the endoplasmic reticulum. That the rapidly labeled peak is not as sharp as that seen by Warner *et al.* (1962, 1963) in reticulocytes, may be due to polysomal degradation, insufficient removal of lipid contamination or, alternatively, to different molecular size polysomes which exist physiologically. This might be related to the greater variety of proteins being synthesized by HeLa cells growing *in vitro*, in comparison with the more limited repertoire of the rabbit reticulocyte.

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