THE LIFETIME OF MESSENGER RNA FOR COLLAGEN AND CELL PROTEIN SYNTHESIS IN AN ESTABLISHED MAMMALIAN CELL LINE

Sherman Bloom, Burton Goldberg and Howard Green

Department of Pathology

New York University School of Medicine, New York, New York

Received March 4, 1965

Measurements of messenger RNA decay based on rates of protein synthesis or on polyribosome stability have given quite similar results. In bacteria, messenger RNA is characterized by a half-life of a few minutes (Levinthal, Keynan and Higa, 1962; Gros et al, 1963; and Kepes, 1963). In the case of animal cells, the turnover rate of messenger RNA is much slower. Messenger RNA with a half-life of 2-3 hours has been reported for HeLa cells growing exponentially in culture (Penman, Scherrer, Becker and Darnell, 1963), mouse liver (Trakatellis, Axelrod and Montjar, 1964), and embryonic chick lens (Scott and Bell, 1965). Much longer-lived messengers have been reported for reticulocytes (Marks, Burka and Schlessinger, 1962), down feather cells (Humphreys, Penman and Bell, 1964) and chick embryo lens (Scott and Bell, 1964), all of which are tissues or cells committed to the production of large amounts of a limited variety of specific proteins. Clearly in the case of the reticulocyte, and probably in the other instances (Scott and Bell, 1964) of long-lived messengers, the cell populations are effectively anucleate. It has been reported that lamb thyroid, presumably capable of continued RNA and DNA synthesis, possesses both short and long-lived messengers (Seed and Goldberg, 1963).

3T6 is a homogeneous established mouse fibroblast line every cell of which is capable of collagen synthesis (Goldberg and Green, 1964). About 7%

of the total cell protein being synthesized at any given time in stationary cultures is collagen. Such cells will resume exponential growth when subcultured at low cell densities. We have examined the rate of C^{14} Proline incorporation into protein in stationary phase cultures of 3T6 in the presence of actinomycin D to compare the stability of the messenger-RNA for collagen with that of the messengers for the other proteins synthesized by the cells.

Franklin (1963) has studied in detail the in vitro action of actinomycin D on logarithmically growing L cells. He has shown that with concentrations of the antibiotic between 0.5 and 2.5 μg/ml., the synthesis of all species of RNA is halted, with the exception of the 4S RNA, whose synthesis is partially inhibited. The results were very similar in our system of stationary 3T6; concentrations of actinomycin of 1-2 μg/ml reduced the total incorporation of tritium-labelled uridine into RNA to 2-3% of the control values.

To measure the stability of messenger-RNAs in 3T6, cultures were grown to saturation density, and actinomycin 1.0 μg/ml was added in fresh medium. At various times thereafter uniformly C^{14} labelled L-Proline was added and allowed to remain for 3 hours. Activity was then stopped with trichloracetic acid, unlabelled proline and hydroxyproline added and the products exhaustively dialyzed. The non-dialyzable material was then hydrolyzed in 6 N HCl for 12 hours at 120° C. Proline and hydroxyproline were separated by thin layer chromatography (Myhill and Jackson, 1962) and eluted from the cellulose with hot absolute methanol. Aliquots of these eluates were counted by liquid scintillation and the radioactivities corrected for total recovery after quantitative analysis for proline (Chinard, 1952) and hydroxyproline (Prockop and Udenfriend, 1960). The C^{14} incorporated as hydroxyproline represents collagen synthesis, while C^{14} incorporated as proline represents mainly non-collagen "cell protein" synthesis (Green and Goldberg, 1964).

Fig. 1 shows that from the time of addition of actinomycin, the rate of proline incorporation declined in a strictly exponential fashion, with a half-life of 3 hours. The rate of hydroxyproline incorporation, aside from a slight "shoulder" at the beginning, also declined exponentially with the same half-life. As the curves are exponential and of identical slope, all the messengers being measured are homogeneous with respect to lifetime. A similar half-life for collagen messenger and other protein messengers in organ cultures of embryonic chick tibias has been obtained independently by Jeffrey (1965).

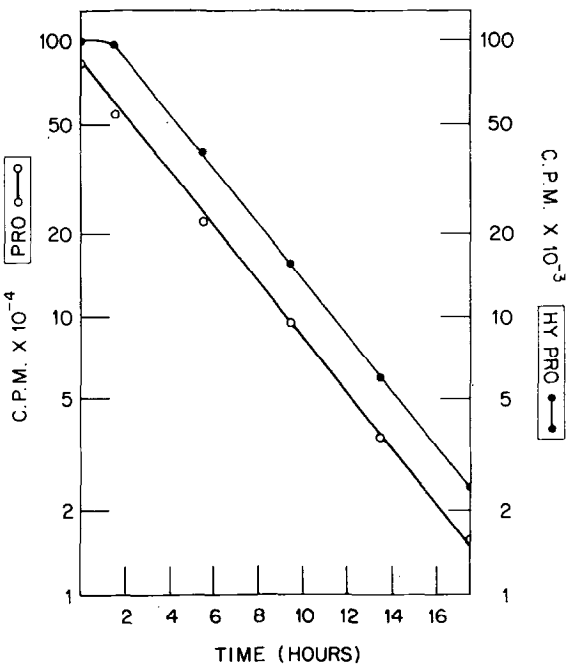

Figure 1. Effect of Actinomycin on the rate of cell protein and collagen synthesis.

Six cultures of 3T6 were grown to saturation density (10^7 cells/20 cm^2) in Petri dishes containing Dulbecco and Vogt's modification of Eagle's medium (1957) supplemented with 10% calf serum and 50 μg/ml. of sodium ascorbate. Five cultures received actinomycin (1 μg/ml). The control plate (no actinomycin) and one of above plates received 5 μC of uniformly labelled C^{14} proline at zero time. The same amount of labelled proline was added to the remaining cultures at 4, 8, 12 and 16 hours respectively. In each case activity was stopped with trichloracetic acid (final concentration 5%) after 3 hours incubation with label. Proline and hydroxyproline were isolated from the labelled proteins and their isotope concentrations determined. Points are plotted at the middle of each three hour incubation period (Ordinates, log scale).

Collagen is a protein with an unusual amino acid composition and a rather large molecular weight. It is synthesized on the rough-surfaced endoplasmic reticulum and is destined for secretion from the cell (Goldberg and Green, 1964); yet its messenger has the same lifetime as that of all the other messengers. In this instance it appears that the intracellular environment determines the messenger half-life, not the nature of the messenger itself. Where all messengers in a given cell type have the same half-life, quantitative regulation of protein synthesis need only depend upon the relative numbers of different messenger molecules and/or the frequency of reading of each messenger. This seems to be the case for bacteria (Attardi et al, 1963). The reported instances of coexistent messengers of different half-lives in animal tissues may prove to be due to mixed cell populations.

References

Attardi, G., Naono, S., Rouvière, J., Jacob, F. and Gros, F. (1963) Cold Spring Harbor Symposia on Quantitative Biology, 28, 363.

Chinard, F.P. (1952) J. Biol Chem., 199, 91.

Eagle, H., Oyama, V.I., Levy, M. and Freeman, A.E. (1957) J. Biol. Chem., 226, 191.

Franklin, R. (1963) Biochem. Biophys. Acta, 72, 555.

Goldberg, B. and Green, H. (1964) J. Cell Biol., 22, 227.

Green, H. and Goldberg, B. (1964) Nature, 204, 347.

Gros, F., Dubert, J., Tissières, A., Bourgeois, S., Michelson, M., Soffer, R. and Legault, L. (1963) Cold Spring Harbor Symposia on Quantitative Biology, 28, 299.

Humphreys, T., Penman, S. and Bell, E. (1964) Biophysical Society Abstracts, 8th Annual Meeting.

Jeffrey, J.J. (1965) Thesis, Georgetown University.

Kepes, A. (1963) Biochem. Biophys. Acta, 76, 293.

Levinthal, C., Keynan, A. and Higa, A. (1962) Proc. Nat. Acad. Sci. U.S., 48, 1631.

Marks, P., Burka, E. and Schlessinger, D. (1962) Proc. Nat. Acad. Sci. U.S., 48, 2163.

Myhill, D. and Jackson, D. (1963) Anal. Biochem., 4, 400.

Penman, S., Scherrer, K., Becker, Y. and Darnell, J. (1963) Proc. Nat. Acad. Sci. U.S., 49, 654.

Prockop, D. and Udenfriend, S. (1960) Anal. Biochem., 1, 228.

Reich, A., Franklin, R., Shotkin, A. and Tatum, E. (1962) Proc. Nat. Acad. Sci. U.S., 48, 1238.

Scott, R. and Bell, E. (1965) Science, 147, 405.

Seed, R. and Goldberg, I. (1963) Proc. Nat. Acad. Sci. U.S., 50, 275.

Trakatellis, A., Axelrod, A. and Montjar, M. (1964) J. Biol. Chem., 239, 4237.