Acta Medica Okayama

Volume 17, *Issue* 5 1963 *Article* 5

1963

Does reticulocyte synthesize RNA ?

Satimaru Seno[*] Masanobu Miyahara[†] Osamu Ochi[‡]

Kenichi Matsuoka[**] Yuki Toyama[††] Takashi Shibata[‡‡]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,

Does reticulocyte synthesize RNA ?*

Satimaru Seno, Masanobu Miyahara, Osamu Ochi, Kenichi Matsuoka, Yuki Toyama, and Takashi Shibata

Abstract

As has been well established, reticulocytes (RC) synthesize the species specific protein, globin, actively for about 24 hours or more till the time of their complete maturation1,2,3. This will be possible only in the presence of messenger RNA (m-RNA)4,5. Since the splendid hypothesis of m-RNA proposed by JACOB and MONOD6 for explaining the mechanism of the transfer of genetic information from nucleus to cytoplasm, it has largely been accepted through the numerous observations that followed7,8,9,10. However, the m-RNA hypothesis, which has been deduced by observing the protein synthesis in E. Coli, includes the meaning of labile RNA which is incessantly decomposed and newly synthesized to compensate the rapid degradation. As m-RNA cannot be synthesized in RC which have no detectable DNA, it has been supposed that the m-RNA of RC should be considerably stablell,12,13. Even in the denucleated cells, however, the RNA synthesis might be possible because Borsook reported the positive RNA synthesis of RC14, and this result has recently been reconfirmed by BURNY15.

*PMID: 14164122 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 17, 253—256 (1963)

BRIEF NOTES

DOES RETICULOCYTE SYNTHESIZE RNA ?

Satimaru SENO, Masanobu MIYAHARA, Osamu OCHI,
Kenichi MATSUOKA, Yuki TOYAMA and Takashi SHIBATA

Department of Pathology, Okayama University Medical School
(Director : Prof. S. Seno)

Received for publication, August 2, 1963

As has been well established, reticulocytes (RC) synthesize the species specific protein, globin, actively for about 24 hours or more till the time of their complete maturation[1,2,3]. This will be possible only in the presence of messenger RNA (m-RNA)[4,5]. Since the splendid hypothesis of m-RNA proposed by JACOB and MONOD[6] for explaining the mechanism of the transfer of genetic information from nucleus to cytoplasm, it has largely been accepted through the numerous observations that followed[7,8,9,10]. However, the m-RNA hypothesis, which has been deduced by observing the protein synthesis in *E. Coli*, includes the meaning of labile RNA which is incessantly decomposed and newly synthesized to compensate the rapid degradation. As m-RNA cannot be synthesized in RC which have no detectable DNA, it has been supposed that the m-RNA of RC should be considerably stable[11,12,13]. Even in the denucleated cells, however, the RNA synthesis might be possible because Borsook reported the positive RNA synthesis of RC[14], and this result has recently been reconfirmed by BURNY[15].

For the purpose to look into the RNA synthesis in RC the authors observed the rabbit RC incubated with one of the precursors of RNA, H^3-uridine. And it has been revealed by the radioautographic method that no incorporation of the urindine into the RNA of reticulocyte does occur suggesting that not any kind of RNA can be synthesized in RC.

As the material the rabbit reticulocytes were used. A male rabbit weighing 2,800 g (RBC: 6.7 millions per cu mm, Ht: 43%, and RC: 2.3%) received 6.0 ml of 2.5% neutralized phenylhydrazine hydrochloride in aqueous solution being injected subcutaneously in the dose of 1.5 ml once a day for 4 days. Four days after the last injection 10 ml of blood was taken from the ear vein. Red cells having Heinz bodies disappeared within these four days and the RC were 57 per cent of RBC, which were 2.53 millions per cu mm with Ht 25.3 per cent. The blood was taken with the syringe containing 0.8 ml of 3.8% sodium citrate to prevent the coagulation. Five ml of citrated blood was combined with

some Ehrlich ascites tumor cells by mixing with 1 ml of fresh ascites from the mouse bearing the tumor ascites fluid. The tumor cells served to check the incorporation of H^3-uridine into the RNA of cytoplasm of living nucleated cells in the medium used for the incubation. After adding the tumor cells the blood was centrifuged at 4, 000 r.p.m. for 10 min and the packed cells mixed with the tumor cells were obtained. 0.2 ml of the packed cells was resuspended in 7 ml of normal rabbit plasma. 0.2 ml of this suspended sample was added to 0.04 ml of Gey's solution and 0.01 ml of H^3-uridine to make the final concentration of the uridine to be 1 μc per ml of the incubation mixture. 0.25 ml of the red cell suspension was taken into the test tube of 1.6 cm in diameter and incubated at 37°C for 1.5 hours, shaking gently on the water bath. The gas phase for the incubation was air. After 1.5 hr incubation the cells were washed with 7 ml of cold rabbit plasma twice by repeating centrifugation, at 3, 000 r. p. m. for 5 min each. After washing the cells were resuspended in 1 ml of cold rabbit plasma. The cells were stained supravitally on the object glass coated with a dye film of Nile blue by the routine method. The dye film was prepared by smearing 0.1% Nile blue solution in pure ethanol. After about 20 sec staining, the cells were smeared, dried and fixed for 30 sec with methanol. The free H^3-uridine in the cells was removed by treating the smears with cold 0.02 N perchloric acid (0°C) for 5 min. Through this treatment the hemoglobin was removed but the reticulocytes could be recognized by their blue-stained cytoplasm or granulo-filamentosa. Then the smears were washed with distilled water and dried, and covered with AR 10 Kodak radioautograph film for stripping method[16]. These smears were kept in a dark room for 20 days in a refrigerator. After 20 days' exposure the films on the smears were developed and the cells were stained with Giemsa.

Observations revealed no grains on the reticulocytes, while a mass of grains on the Ehrlich ascites tumor cells as can be seen in Fig. 1. The similar observation on the reticulocytes from another rabbit invariably showed no grains on the reticulocytes.

The foregoing result shows clearly that the reticulocytes have no ability to incorporate the given uridine into the RNA nor any indication of new synthesis of RNA to be proceeding in the reticulocyte. This fact seems to show that not any kind of RNA can be synthesized without nucleus or DNA. The incorporation of the C^{14}-glycine[14] or C^{14}-guanine into the RNA of reticulocyte[15], as observed by the biochemical analysis *in vitro*, is probably due to the contamination on the nucleated cells in the fraction of reticulocytes. RNA synthesis in the denucleated amebae[17] or algae[18] has been reported but in these cases, too, it seems to be unlikely as has been pointed out by Prescott[19], and suggests no synthesis of RNA in the absence of DNA. Thus all the RNA found in the reticulocyte seems to have been formed in the erythroblastic stage and is destined to be decomposed

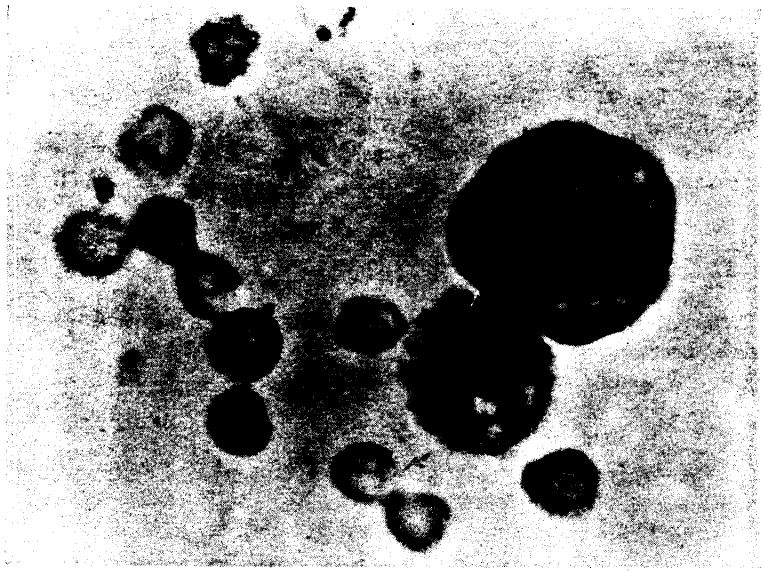

Fig. 1 Radioautograph of the rabbit reticulocytes and Ehrlich ascites tumor cells, incubated with H^3-uridine (1 μc/ml) for 1.5 hr. The picture shows the incorporation of H^3-uridine into RNA of tumor cells but not of reticulocytes. For the method refer to the text.

only with the advance of protein synthesis. Therefore, m-RNA in reticulocytes, which is also formed in the nucleated stage, should be of a lasting one differing from that found in coli bacteria. The result supports the observation of MUNRO and CORNER[20], which revealed no inhibitory effect of actinomycin D on protein synthesis in reticulocyte.

REFERENCES

1. LAMFROM, H.: *Fed. Proc.* 19, 350, 1960
2. LIPMANN, F. and von EHRENSTEIN, G.: *Proc. Natl. Acad. Sci.* 47, 941, 1961
3. BISHOP, J., FAVELUKES, G., SCHNEET, R, and RUSSEL, E.: *Nature* 192, 1365, 1961
4. WATSON, J. D.: *Science* 140, 171, 1963
5. MARKS, P. A., RIFKIND, R. A. and DANON, D.: *Proc. Natl. Acad. Sci.* 50, 3361, 1961
6. JACOB, F. and MONOD, J.: *J. Mol. Biol.* 3, 318, 1961
7. CRICK, F. H. C., BARNETT, L., BRENNER, S. and WATTS-TOBIN, R. J.: *Nature* 192, 1227, 1961
8. DINTZIS, H. M.: Proc. *Natl. Acad. Sci.* 47, 247, 1961
9. BRENNER, S., JACOB, F. and MESELSON, M.: *Nature* 190, 576, 1961
10. GROS, F. H., HIATT, H., GILBERT, W., KURLAND, C. G., RISEBROUGH, R. W. and Watson, J. D.: *Nature* 190, 581, 1961
11. NATHAN, D., von EHRENSTEIN, G., MUNRO, R. and LIPMANN, F.: *Fed. Proc.* 21, 127, 1962

12. MARKS, P. M., BURKA, E. R. and SCHLESSINGER, D.: *Proc. Natl. Acad. Sci.* 48, 2163, 1962
13. REICH, E., FLANKLIN, A. J., SHUTKIN, A. J. and TATUM, E. L.: *Proc. Natl. Acad. Sci.* 48, 1238, 1962
14. KRUCH, J. and BORSOOK, H.: *Nature* 175, 386, 1955
15. BURNY, A.: *Arch. Internal. Physiol. et. Biochim.* 70, 742, 1962
16. FITSGERALD, P. J., SIMMEL, E., WENSTEIN, J. and MARTIN, C.: *Lab. Invest.* 2, 181, 1953
17. PLANT, W., and RUSTAD, R. C.: *J, Biophys. Biochem. Cytol.* 3, 3, 625, 1957
18. BRACHET, J., CHANTRENNE, H. and VANDERHAEGE, F.: *Biochim. et Biophys. acta* 18, 544, 1655
19. De ROBERTIS, D. E. P., NOWINSKI, W. W. and SAE Z, F. A.: General Cytology, p. 313, Saunders, London, 1960
20. MUNRO, A. J. and CORNER, A.: *Nature* 197, 891, 1963