ASSOCIATION OF RETICULOCYTE RNA WITH THE CELL MEMBRANE

E. Burka, W. Schreml and C. Kick

The Cardeza Foundation for Hematologic Research
Jefferson Medical College
Philadelphia, Pennsylvania

Received December 30, 1966

The mammalian reticulocyte does not synthesize RNA (Marks, Burka and Schlessinger, 1962), but progressively loses RNA as the cell matures to become an erythrocyte (Bertles and Beck, 1962). The present studies were undertaken to determine whether the reticulocyte membrane participates in the metabolism of erythroid ribonucleic acid. The findings show that a significant proportion of total reticulocyte RNA is closely associated with the cell membrane in a manner which is not dependent upon the structural or metabolic integrity of the membrane. The metabolism of the RNA which is membrane-associated is different than that of the RNA which exists free within the interior of the cell. The data indicate that the erythroid cell membrane does have a specific role in the nucleic acid metabolism of the erythroid cell.

Peripheral erythroid cells, obtained from normal rabbits or animals with a phenylhydrazine-induced reticulocytosis (Borsook, Fisher and Keighley, 1952), were washed and lysed with 20 milliosmolar phosphate buffer as described by Dodge, Mitchell and Hanahan (1963). The amount of RNA was determined in crude whole lysates, in membrane-free lysates and in sedimented washed membranes by the acid extraction method (Burka, 1966) or following isolation and purification by the phenol technique (Marks, et al., 1962). Sucrose density gradient centrifugation and determination of radioactivity were done as described previously (Marks, et al., 1962).

The proportion of total cellular ribonucleic acid and hemoglobin which was

associated with erythroid cell membranes following lysis and washing in hypotonic phosphate buffer is shown in Figure 1. Repeated washing of the membranes

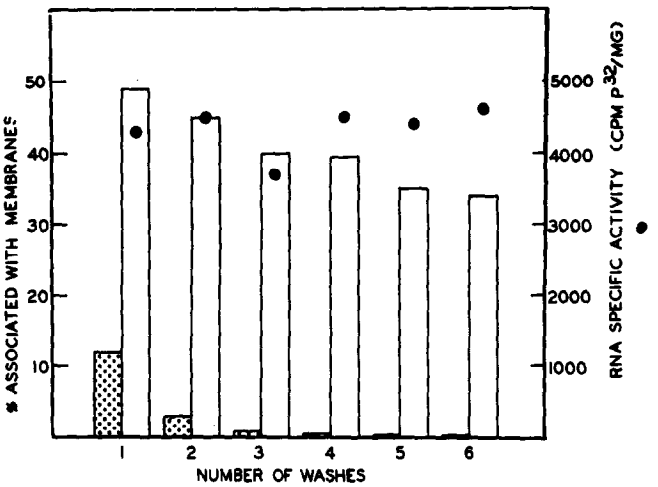

FIGURE 1. Proportion of total cellular RNA (open bars) and hemoglobin (dotted bars) associated with reticulocyte membranes following lysis and washing in 20 milliosmolar phosphate buffer. The solid circles indicate specific activity of the P^{32}-labelled RNA.

reduced the proportion of total cellular hemoglobin to less than 0.4% of that originally present, as determined by the cyanmethemoglobin method. Although there was a slight reduction in the amount of RNA associated with the membranes during the washing procedure, a large proportion of total cellular RNA remained closely associated with the membranes. The RNA of the cells used in this experiment had been previously labelled in vivo with P^{32} (DeBellis, Gluck and Marks, 1964). The specific activity of the RNA associated with the membrane remained constant during the washing procedure. These findings indicate that a significant proportion of erythroid cell RNA, of constant composition, is closely bound to erythroid cell membranes which are essentially hemoglobin free. Quantitatively similar results were obtained when RNA associated with the membrane was determined following phenol isolation and purification. The degree of association showed neither positive nor negative correlation with the degree of reticulocytosis. In 34 samples of peripheral blood, with reticulocytosis varying from

2 to 99%, the proportion of total cellular RNA which was associated with the cell membrane averaged 31.1 ± 8.9%.

Studies were done to determine whether the amount of membrane associated RNA would vary depending on the method of cell lysis. The amount of membrane-bound RNA, when compared with that found following the standard method of hypotonic lysis, was not altered when cells were lysed by repeated freezing and thawing, a procedure which fragments the membrane, or by exposure to 0.4 molar butanol or to saponin, agents which disrupt the phospholipid organization of the membrane. These data indicate that the RNA associated with the membrane is not merely trapped within the red cell ghost, nor does the association depend upon the gross structural or metabolic integrity of the membrane.

The composition of P^{32}-labelled reticulocyte RNA, extracted separately from the cell membranes and from membrane-free hemolysates by the phenol method, was examined by sucrose density gradient centrifugation. As shown in Figure 2, the three usual molecular components of mammalian RNA are present in each of the

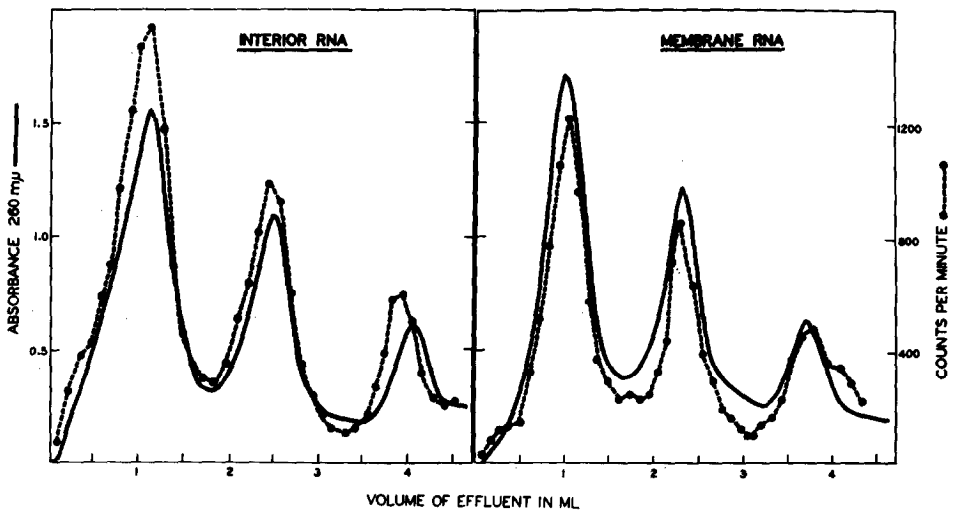

FIGURE 2. Sucrose density gradient centrifugation patterns of free and membrane-associated reticulocyte RNA extracted 40 hours after *in vivo* labelling with P^{32}. Centrifugation was for 4 hours at 39,000 rpm (Spinco SW39 rotor) through a 4.8 ml 5 to 20% linear sucrose gradient in 0.1M acetate buffer, pH 5.0. Absorbance was read in a Gilford model 2000 recording spectrophotometer and 2 drop samples collected for determination of radioactivity.

isolated fractions of reticulocyte RNA. The proportion of each of these molecular components in each of the two fractions of erythroid RNA is the same, transfer RNA representing approximately 18% of the total. The specific activities of the two fractions of RNA, sampled 40 hours after in vivo pulse labelling, are not similar, as indicated by the relative positions of the lines indicating radioactivity and RNA concentration throughout the gradient (Figure 2). The specific activity of the free RNA in the interior of the cell is distinctly greater than that of membrane-bound RNA. Studies of the turnover of the two fractions of erythroid cell RNA, using either inorganic P^{32} or C^{14} uridine as a precursor, indicated that at the time of maximum labelling in the peripheral blood, 40 hours after pulse labelling in vivo (DeBellis, et al., 1964) the specific activity of the membrane-bound RNA was consistently about 75% that of the value reached by the free RNA. These findings indicate that newly synthesized erythroid RNA makes its appearance in the membrane-bound fraction at a slower rate than it does in the free fraction.

Membrane-bound RNA and ribosomes are important in protein biosynthesis (Hendler, 1965). These studies indicate that a significant proportion of mammalian reticulocyte RNA is intimately associated with the cell membrane. Although the molecular components of the two fractions of reticulocyte RNA are similar, their rates of turnover appear to be different. The data are consistent with either different rates of synthesis of the two fractions of erythroid RNA *in situ*, or a continuing unequal exchange between the free and membrane-bound RNA. The presence of a fraction of erythroid RNA which is membrane-bound and which differs in metabolic activity from the RNA found in the interior of the cell suggests that the cell membrane may have an important role in determining the rate of protein synthesis in the maturing mammalian erythroid cell.

These studies were supported by USPHS Grant HE-10473. Dr. Schreml is a Fellow of the Deutsche Forschungsgemeinshaft.

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