RAT LIVER AND HEPATOMA POLYSOME-MEMBRANE INTERACTION IN VITRO[1]

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Ultrastructural studies of rat liver have shown that the endoplasmic reticulum (ER) consists of at least two forms, the rough form, composed of membranes with ribosomes attached to their surface, and the smooth form, composed only of membranous vesicles of various sizes (Palade and Siekevitz, 1956). By means of isopycnic sucrose gradients it is possible to separate rough and smooth ER (Rothschild, 1961). Furthermore by use of chelating agents, one can remove many of the ribosomes attached to the surface of the membranes of the rough ER (Sachs, 1958, Vogt, 1960). On the other hand, by the use of detergents and discontinuous gradients, operational separation of "free" and "bound" polysomes and ribosomes may be accomplished (Webb, et al., 1964). Webb et al. (1965) have reported that the proportion of free and bound polysomes is quite different in most hepatomas when compared with normal liver in vivo. The present communication describes experiments on the recombination in vitro of polysomes and ER membranes isolated from liver and hepatoma.

MATERIALS AND METHODS

Smooth and rough membranes of rat liver were prepared according to Shapot and Pitot (1966), using 1.23 M sucrose for floating smooth membranes.

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A Spinco model L2 type of centrifuge was used for all centrifuge procedures. The membranes were washed in 0.01 M sodium pyrophosphate, pH 7.4, according to the method of Sachs (1958) and twice in 0.2 M sucrose. Membranes from Novikoff hepatomas (solid form) were prepared from the microsomal pellet (2 hrs centrifugation at 30,000 rpm) by flotation in 1.31 M sucrose. They were washed with pyrophosphate as above. Ribosomes were removed from the rough ER as follows: The rough membranes were rehomogenized in 0.25 M sucrose containing 0.08 M sodium citrate pH 7.4 (Vogt, 1960), 0.01 M sodium pyrophosphate pH 7.4 (Sachs, 1958) and 0.01 M sodium-potassium phosphate pH 7.4 (1 ml. for membranes from 5 gms of liver). After 30 min at $0^{o}C$ the suspension was layered over 10 ml of 1.7 M sucrose and spun for 16 hrs. at 30,000 rpm. The membranes floating over 1.7 M sucrose were collected, treated once more with citrate and pyrophosphate and spun for 35 min at 30,000 rpm. The pellets were washed with 0.2 M sucrose and stored in 0.2 M sucrose at $-18^{o}C$. These membrane fractions are referred to in this paper as "stripped". For the preparation of P^{32} labeled polysomes, each animal was injected intraperitoneally with 1.0 mc $Na_3P^{32}O_4$ and killed 24 hours later. Polysomes were prepared as recently described (Webb, et al., 1964). The separation of free and membrane-bound polysomes was based on the finding that essentially all bound polysomes of a deoxycholate-treated postmitochondrial supernatant sedimented through a double layer of sucrose (3 ml of 2.0 M overlayered by 4 ml of 0.5 M) prepared in 0.05 M tris, 0.025 M KCl and 0.005 M $MgCl_2$, pH 7.5 in 30 minutes at 105,000 xg with virtually no contamination by free polysomes (Blobel and Potter, unpublished). Free polysomes not bound to membranes were obtained by centrifuging the postmitochondrial supernatant for 24 hours under the same conditions but in the absence of any detergent.

Rehomogenized membranes (0.5 and 2.5 mg protein) and P^{32} labeled polysomes (50 micrograms RNA), prepared by the method of Webb et al. (1964),

were added to 2.0 ml 0.2 M sucrose containing 0.01 M Tris pH 7.4 and 0.005 M $MgCl_2$ at 4^oC. The same buffer was added to a final volume of 5 ml and the mixture vigorously shaken for 20 seconds. After 30 min at 0^oC, 4 ml of these suspensions was layered over 1 ml of 2.0 M sucrose (previously treated with Bentonite), in 0.01 M Tris, 0.005 M $MgCl_2$ and centrifuged for 16 h at 39,000 rpm in a swinging bucket. The supernatant containing the floating membranes was decanted from the polysome pellet, diluted with 6 ml H_2O and spun for 40 min. at 40,000 rpm. The resulting membrane pellet as well as the polysome pellet were each dissolved in 0.5 ml Hyamine and counted in a Packard Tricarb scintillation counter.

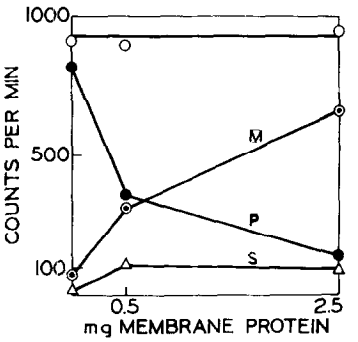

Figure 1. In vitro combination of polysomes and membranes. P = counts measured as "free" polysomes; M = counts measured as "recombined" polysome-membrane complex and S = counts in soluble supernatant. The line at 900 cpm parallel to the abscissa indicates the recovery of added counts in the three fractions. The deviation of duplicates was less than 10%.

RESULTS AND DISCUSSION

A balance-sheet of a recombination experiment is given in fig. 1. P-32 labelled polysomes were mixed with two different amounts of membranes. Free and "recombined" polysomes were separated by centrifugation over 2.0 M sucrose. As can be seen, 0.5 mg of membranes associated with about 50% of the polysome counts and increasing the amount of membranes five times only

resulted in a doubling of the combination of P^{32} and membrane. Washing of the polysome-membrane complex formed in vitro in 0.2 M sucrose for 30 minutes at 100,000 xg did not solubilize measurable amounts of radioactivity. Fig. 2 shows a recombination experiment using different membrane

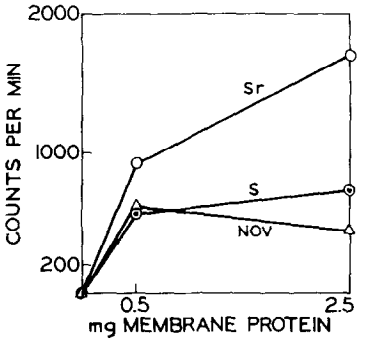

Figure 2. In vitro combination of polysomes and membranes from "stripped" membranes (Sr), smooth ER (S) from liver and ER prepared from Novikoff hepatoma (NOV). Only the counts "recombined" as the polysome-membrane complex are seen in this figure.

preparations. "Stripped" rough membranes from rat liver appeared to be more effective in combining with rat liver polysomes than either smooth membranes from liver or membranes prepared from the Novikoff hepatoma. In a similar experiment with polysomes prepared from Novikoff hepatoma results identical to those seen in Fig. 2 were obtained. These results indicated that the differences noted in the association of polysomes and various membrane fractions resulted from various membrane structures rather than differences in the source of the polysomes. The lesser association of polysomes with smooth membranes may have resulted from nonspecific binding of the polynucleotide-ribosome-complex to the membrane surface under the experimental conditions. Both types of ER membranes, smooth as well as "stripped" rough, have a negative overall charge at pH 7 as shown by the binding of an acidophilic dye (Süss, unpublished). The recombination of negatively charged polysomes with negatively charged

membranes therefore cannot be regarded as "neutralization" of a polycation with a polyanion. The observation that Novikoff membranes bind polysomes as little as smooth-liver membranes is in agreement with the finding that the Novikoff hepatomas contain only a few polysomes attached to the endoplasmic reticulum as seen in the electron microscope (Howatson and Ham, 1955). The binding of free and bound polysomes from rat liver to "stripped" rough membranes is shown in fig. 3. Both types of polysomes were bound to the same extent under these experimental conditions.

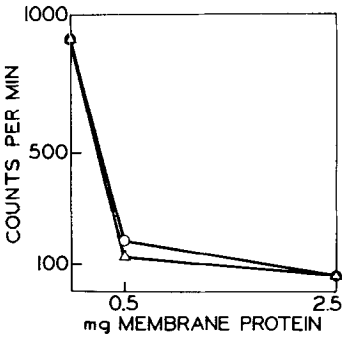

Figure 3. In vitro combination of free (O) and bound (Δ) polysomes to "stripped" membranes. Only the counts measured as remaining "free" polysomes after recombination in vitro are shown here.

These experiments provide confirmation in vitro of the in vivo studies of Webb et al. (1965) and indicate that free and bound polysomes are not significantly different as measured by this criterion. Thus they may represent different metabolic states of the same functioning unit. Furthermore, it would appear that the formation of the rough ER, i.e. association of polysome and membrane, is directed in part by the membrane, possibly by "combining-sites" present in the membranes of the rough ER which are missing or "suppressed" in those of the smooth ER. The number of available binding sites would thus determine the amounts and/or the kind of bound polysomes. Changes in the number of availability of binding

sites in the membrane would then lead directly to changes in the ratio of free to bound polysomes, a ratio which seems to be characteristic for different stages of differentiation as shown for growing and resting cells in tissue culture (Levine, et al., 1965). In addition alterations in membrane structure may be responsible for altering the population of bound polysomes as well as the degree of binding of the population. Such an alteration has been suggested to play a key role in the malignant transformation (12).

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