Review Letter

FUNCTIONS OF POLYRIBOSOMES ATTACHED TO MEMBRANES OF ANIMAL CELLS *

P.N.CAMPBELL

Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds, L52 9L5, England

Received 30 Januaary 1970

I propose first to consider some advantages to the physiology of the cell to be gained from the attachment of polysomes to the membrane of the endoplasmic reticulum. Later I consider whether all the proteins synthesized in a cell which is richly endowed with endoplasmic reticulum are synthesized by the membrane-bound polysomes.

Fig. 1 reminds us that according to present concepts the nascent polypeptide chain is extruded into the cisternae formed by the membrane of the endoplasmic reticulum. The precise nature of the attachment of the large subunit to the membrane is not clear at present.

1. Advantage of attachment of polysomes to membranes

A major advantage to the cell to be gained from the attachment of polysomes to membranes arises if the nascent protein is destined to be transported elsewhere in the cell. From the work of Peters [1] on the synthesis of serum albumin by liver cells under *in vivo* conditions and from our own work on protein synthesis by the isolated microsome fraction [2], it has been assumed that the newly synthesized albumin is contained within the membrane of the reticulum. Serum albumin has been taken as a model for the synthesis of all serum proteins made by the liver. Peters [3] has also shown that a protein such as catalase, a component of the microbodies, is synthesized by the membrane-bound polysomes. More recently the site of synthesis of the soluble protein in the mitochondria has attracted attention. Cytochrome *c*, which may be typical of such proteins, has been shown to be synthesized by the polysomes in the cytosol but it has not proved possible to locate its site of synthesis more precisely [4]. Nevertheless it would not be surprising if the soluble protein of the mitochondria were to be found to be synthesized on the membrane-bound polysomes.

Two further examples of the advantage of containing the newly synthesized protein within a membrane may be mentioned. Brew [5] showed that extracts of guinea-pig lactating mammary gland effected the synthesis of the whey protein, α-lactalbumin. He showed that this small protein, molecular weight about 15000, was retained by the vesicles of the endoplasmic reticulum from which it could be released by ultrasonic vibrations. Ebner [6] later showed that the synthesis of lactose involved two proteins, the so-called A and B proteins of lactose synthetase, and that α-lactalbumin was the B protein. Brew et al. [7] then at Duke University, showed more precisely how α-lactalbumin acted in the synthesis of lactose and the conclusions may be summarised as follows:

(1) UDP-galactose + *N*-acetylglucosamine $\xrightarrow{\text{A protein}}$ *N*-acetyllactosamine + UDP

(2) UDP-galactose + glucose $\xrightarrow{\text{A + B proteins}}$ lactose + UDP

The A protein is a transferase which plays an important part in the serial attachment of monosaccharides

* The substance of paper given at a colloquium of The Biochemical Society, Leeds January 1970.

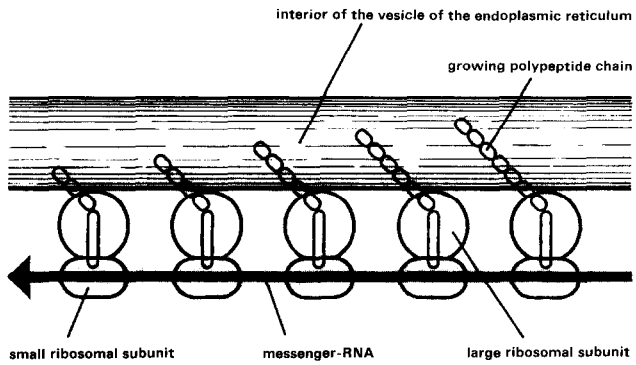

Fig. 1. The structure of the rough-surfaced endoplasmic reticulum.

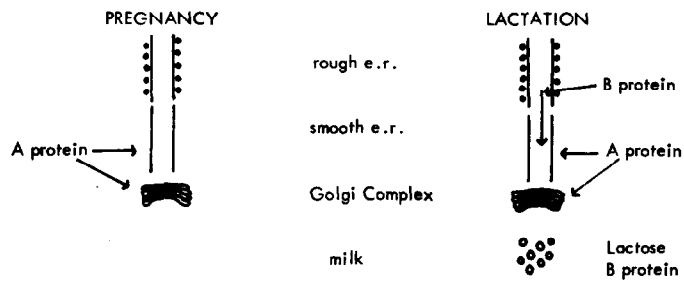

Fig. 2. The role of membrane-bound polysomes in the synthesis of α-lactalbumin and the control of lactose synthesis in the mammary gland. Based on the work of Brew [9].

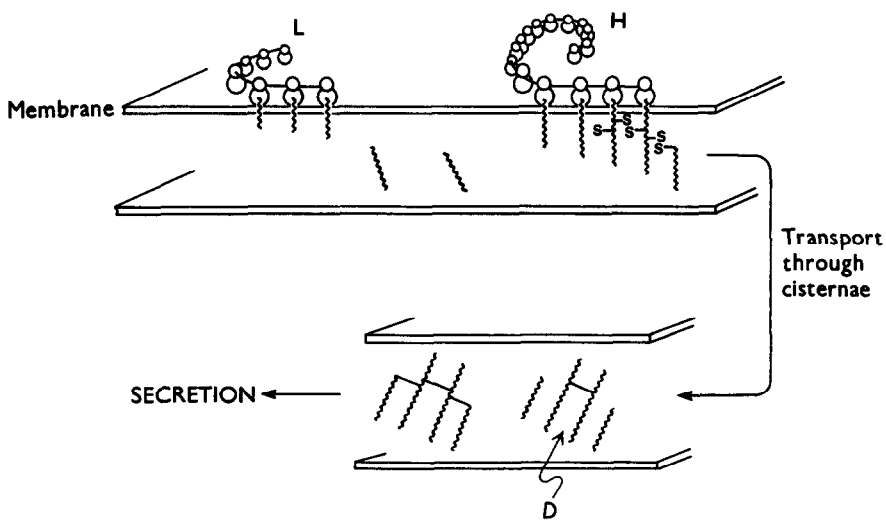

Fig. 3. The role of membrane-bound polysomes in the synthesis of immunoglobulin. From Williamson [10].

to form the carbohydrate moiety of many glycoproteins. The presence of α-lactalbumin (B protein) changes the specificity of the transferase in the presence of substrate glucose and the net result is the synthesis of lactose.

The transferase is located in the membrane of the smooth endoplasmic reticulum, particularly the Golgi Complex [8]. From this and the fact that α-lactalbumin is synthesized by the bound polysomes, Brew [9] has postulated that the lactose synthetase is controlled as shown in fig. 2. As α-lactalbumin passes from the rough to the smooth endoplasmic reticulum its interaction with A protein causes lactose to be synthesized. The passage of α-lactalbumin into the milk is also a useful control mechanism since cessation of the synthetis of α-lactalbumin rapidly stops the synthesis of lactose.

The second example is taken from the work of Williamson and Askonas on the biosynthesis of immunoglobulin [10]. In this multichain protein there is a problem of assembling the two L chains and two H chains into the final product. The chains are, of course, linked through S-S bridges. The present concept is shown in fig. 3. This shows that the two types of chain are synthesized by distinct polysomes, large and small, attached to membranes. More recently Askonas and Williamson [11] have shown that an intermediate is formed, this is shown as D in the figure. The formation of the intermediate and of the finished immunoglobulin is obviously greatly facilitated by the containment of the intermediates within the controlled environment of the cisternal space.

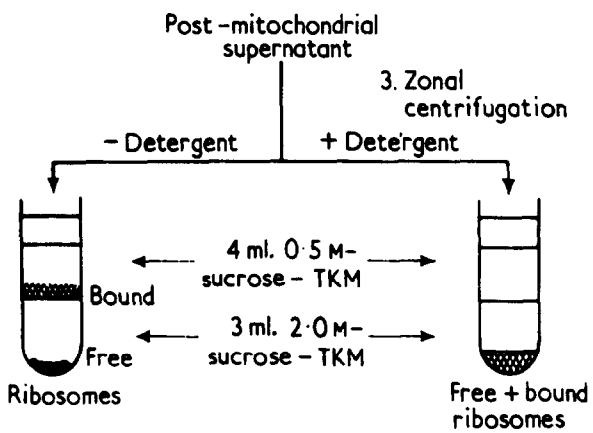

Fig. 4. The preparation from the disrupted liver cell of different polysome-containing fraction. From Blobel and Potter [12].

2. Possible role for free polysomes

It will be clear that the attachment of polysomes, active in the synthesis of protein, to membranes has many advantages for the physiology of the cell. I would now like to turn to a more controversial question. In a cell in which the rough endoplasmic reticulum is highly developed, such as a liver cell from the adult rat, is the protein that is synthesized for the internal economy of the cell also made on membrane-bound polysomes, or is such protein made by polysomes that are not associated with the rough endoplasmic reticulum? In short, does the liver cell contain membrane-free polysomes and, if it does, do these polysomes synthesize proteins which are not made by the membrane-bound polysomes. This has seemed to us an important question, crucial to our understanding of the logistics of protein synthesis in a differentiated animal cell. The idea that there are two kinds of polysomes, the one being concerned with the synthesis of protein for export, the other with the synthesis of retained protein, is attractive for, otherwise, the proteins produced by the endoplasmic reticulum would have to be seggregated in an organelle like the Golgi Complex in order to prevent the retained protein leaving the cell.

In electron micrographs of the liver cell, we can see polysomes which do not appear to be associated with the endoplasmic reticulum but we cannot be sure of this interpretation. We can also isolate from a cell homogenate two fractions which we operationally term bound and free polysomes. Fig. 4 shows a commonly employed method for their preparation taken from the work of Blobel and Potter [12].

So far electron microscopy, analysis of RNA and of protein, and studies on the metabolic turnover of RNA have not revealed any significant difference between the ribosomes in the two fractions. Moreover, there seems little difference in the ability of the polysomes in the two fractions to effect the synthesis of total protein either *in vivo* or *in vitro* nor is there a marked difference in the size of the polysomes in the two fractions. One might conclude, therefore, that the polysomes in the two fractions have a common derivation. According to this the so-called free-polysomes would be

detached from the endoplasmic reticulum during disruption of the cell and subfractionation. The challenge to the biochemist is to demonstrate that the polysomes in the "free polysome" fraction effect the synthesis of a protein that differs from that synthesized by the polysomes in the "bound-polysome" fraction.

3. Problems in studying the synthesis of specific proteins

A major difficulty in this approach is to choose specific proteins the biosynthesis of which can be studied. As already mentioned, serum albumin has been studied as an example of a protein which is exported from the cell. The basic procedure is to study the incorporation of a radioactive amino acid(s) into the purified protein under various conditions. Unfortunately, the isolation of the particular protein in a state of radiochemical purity presents many problems. In our early experiments we showed that the isolation of serum albumin from a crude mixture of proteins by precipitation with a specific antiserum was not in itself a sufficiently critical test of radiochemical purity [13]. This point has been emphasized again more recently by the careful studies of Schreiber and his colleagues in Freiburg [14]. After injection of a rat with ^{14}C-leucine they purified the serum albumin from the mixture of liver proteins. A summary of some of their results is shown in table 1. The percentage of serum albumin in the extracted protein was determined by means of a specific antiserum. It will be seen that not until preparative electrophoresis on polyacrylamide gels had been applied did the specific radioactivity of the serum albumin remain constant. They found that radiochemically pure serum albumin could not be obtained by immunological precipitation alone and conclude that the polyacrylamide electrophoresis step is very important for purification. We do not know, of course, whether the purification of serum albumin poses special problems but these results emphasize how much care must be taken to check the purity of the isolated protein.

4. Studies on the biosynthesis of ferritin and serum proteins

For the study of the site of biosynthesis of retained protein Redman, Munro and his colleagues, have chosen ferritin. Redman [15] compared the synthesis of ferritin and serum proteins under both *in vivo* and *in vitro* conditions but I will only be concerned with the results obtained by *in vitro* studies.

Redman prepared the free and bound polysome fractions essentially as already described (fig. 4) but did not treat either fraction with deoxycholate. After incubation of each fraction with a mixture of ^{14}C-labelled amino acids, puromycin was added and the incubation continued. The effect of puromycin would be to cause the release of the nascent polypeptide chains. The particles were then treated with ultrasonic vibrations which would, in particular, release soluble protein from within the membranous vesicles. Redman

Table 1
Specific radioactivity in protein during the purification of albumin from rat liver after injection of ^{14}C-leucine.

Purification step	Albumin in total protein (%)	Specific radioactivity in protein based on protein estimated by	
		Biuret	Turbidimetry
	Results as dpm/mg protein		
Post mitochondrial supernatant	2.0	3460	
TCA-ethanol, Am-SO_4	25	6210	
Sephadex G-100	28	11200	
DEAE Cellulose	81	12300	10500
Electrophoresis polyacrylamide gel pH 10.3	88		9550
Electrophoresis polyacrylamide gel pH 2.7	92		6880
Charcoal treatment	108		6050

Taken from the work of Schreiber et al. [14].

Table 2
Incubation *in vitro* of free and attached ribosomes with ^{14}C-labelled amino acids and recovery of released proteins with antisera to serum proteins and ferritin.

	Results in disintegrations/mg RNA	
Radioactivity	Free ribosomes	Attached ribosomes
Total protein	80500	136400
Released protein	34600	26800
Serum protein	4440 (13%)*	20250 (75%)
Ferritin	1395 (4%)	202 (1%)

Taken from the work of Redman [15].
* Percentage of radioactivity in released protein.

Table 3
Relative incorporation of ^{14}C-labelled leucine into ferritin and serum albumin by free polysomes and total polysomes from rat liver.

Polysome preparation	Incorporation into specific protein as % of that into total protein	
	Ferritin	Albumin
Total	0.08	0.35
Free	0.13	0.16

Taken from the work of Hicks et al. [16].

then determined the radioactivity of the ferritin and serum proteins in the soluble fraction, i.e. that obtained after treatment with puromycin and ultrasonic vibrations. In some experiments the ferritin was partially purified but with both ferritin and serum proteins specific antiserum was added to precipitate the particular protein required. The precaution was taken of "clearing" the extracts by the prior addition of a protein and its antiserum that did not cross react with rat serum protein.

Some typical results from the work of Redman are shown in table 2. He states that these and the results of other experiments show that the "attached ribosomes make about 6 times more serum proteins than do free ribosomes. Free polysomes, however, synthesize 6 to 20 times more ferritin than do attached ribosomes", So far as the synthesis of serum proteins is concerned it is relevant that the attached ribosome preparation consisted of fragments of the rough endoplasmic reticulum. These will contain large amounts of serum proteins relative to the preparation of free polysomes. As already explained there are good reasons to doubt the effectiveness of an antiserum alone for the separation of the serum proteins and it is very likely that the antibody precipitate was contamined with radioactive substances other than serum proteins. For these reasons the high radioactivity in the serum proteins from the attached ribosomes compared with the free may not be as significant as is claimed by Redman. The presence of membrane in the attached ribosome preparation would not influence the results with ferritin. Provided, therefore, that the radiochemical purity of the isolated ferritin was satisfactory the results of Redman support the more active role of the free polysomes in the synthesis of this protein.

If we turn to the work of Hicks, Drysdale and Munro [16] on the synthesis of serum albumin and ferritin we note that in this case the free polysome preparation

Table 4
Incorporation of ^{14}C-labelled amino acids into protein and purified NADPH-cytochrome *c* reductase by subcellular fractions. (Incubation in presence of cell sap. Radioactivity as cpm/mg RNA incubated).

Stage of purification	Microsomes	Bound polysomes	Free polysomes	Detergent polysomes
Total incubation	6290	6660	11690	30860
Steapsin digest	2410	3050	3290	12510
Steapsin supernatant	155	102	227	720
Reductase after polyacrylamide	4.0	3.9	3.5	6.0

Taken from the work of Ragnotti et al. [20].

was treated with deoxycholate and its activity was compared with that of the total polysomes prepared with deoxycholate. Incubation of the fractions was under conditions that favoured release of nascent protein. Cell sap was added at the completion of incubation and ferritin and serum albumin recovered from the soluble fraction. Ferritin was extensively purified before precipitation with antiserum. The serum albumin was extracted from the cell sap by the trichloroacetic acid-ethanol procedure before the addition of antiserum. The results are summarised in table 3. Once again one doubts whether the methods yielded radiochemically pure serum albumin but at least interpretation of the results is not confused by the presence of membrane in the bound-polysome preparation. The results with ferritin confirm those obtained by Redman and in this case the purification methods were even more rigorous.

Ogata and his colleagues have studied the synthesis of serum albumin by bound and free polysomes both under *in vitro* [17] and *in vivo* [18] conditions. They claim that the synthesis of albumin is confined to the bound polysomes but the methods used for the purification of serum albumin are not adequate. Hallinan et al. [19] have studied the synthesis of glycoprotein using ^{14}C-labelled glucosamine as a precursor. They conclude that the synthesis of glycoprotein is confined to the bound polysomes.

5. The synthesis of membrane protein

It seems right to conclude from this recent work, especially that related to the biosynthesis of ferritin, that the two fractions of polysomes prepared from the disrupted liver cell do have distinct roles in the synthesis of protein in the liver. If this is accepted then it seems correct to conclude that the liver cell of the adult rat contains membrane-free as well as membrane-bound polysomes and that both are active in protein synthesis. The division of labour with respect to protein synthesis between the two types of polysomes is not yet quite clear. In this regard the work of Drs. Ragnotti [20] and Lawford in our laboratory is pertinent. They studied the synthesis of a constitutive membrane protein, NADPH-cytochrome *c* reductase, by various preparations of polysomes and their results are summarised in table 4. Neither the bound polysomes nor the free polysomes were treated with deoxycholate. The microsome fraction was that isolated from disrupted liver by the usual procedure and the detergent polysomes were total polysomes obtained by treatment of the mitochondrial-free supernatant with deoxycholate. The reductase was purified extensively by gel filtration and preparative electrophoresis on polyacrylamide gels. The conclusion was that both bound and free polysomes effected the synthesis of the reductase and that the nascent soluble protein became attached to the membrane of the smooth endoplasmic reticulum. It is tempting to think that the conclusions reached concerning the biosynthesis of reductase may apply to other constitutive proteins of the endoplasmic reticulum.

The work of the laboratories of Schimke [21] and of Siekevitz and Palade [22] on the metabolic turnover of the constituent proteins of the membrane of the endoplasmic reticulum before and after the administration of phenobarbitone to rats, supports the idea that the enzymic composition of the membrane is in a state of flux and that enzymes can be added and deleted from the pre-existing membrane. This would imply that the membranes of the smooth endoplasmic reticulum are able to pick up enzymes made by polysomes that are not associated with membrane, i.e. free polysomes. It is seen, therefore, that the work on the synthesis of reductase by isolated fractions of polysomes is completely in accord with the studies on the metabolic turnover of the membrane-bound enzymes *in vivo*. We conclude, therefore, that in this case the bound and free polysomes effect the synthesis of the same group of proteins.

6. Conclusion

The work reviewed shows that there are important advantages for the physiology of the cell by having polysomes bound to membrane. Present evidence indicates that the adult liver cell contains polysomes, which are active in protein synthesis, that are not attached to the rough endoplasmic reticulum. The synthesis of some proteins appears to be confined to one type of polysome. This conclusion needs to be supported by studies on the synthesis of further proteins. The difficulties inherent in such work have been emphasized.

The presence in the liver cell of two types of poly-

somes presents us with intriguing questions concerning the origin of the messenger RNA and the inter-relationship, if any, between the membrane-free and membrane-bound polysomes.

References

[1] T.Peters jun., J. Biol. Chem. 237 (1962) 1186.
[2] J.R.Sargent and P.N.Campbell, Biochem. J. 96 (1965) 134.
[3] T.Higashi and T.Peters jun., J. Biol. Chem. 238 (1963) 3952.
[4] N.F.González-Cadavid and P.N.Campbell, Biochem. J. 105 (1967) 443.
N.F.González-Cadavid, M.Bravo and P.N.Campbell, Biochem. J. 107 (1968) 523.
[5] K.Brew and P.N.Campbell, Biochem. J. 102 (1967) 265.
[6] U.Brodbeck and K.E.Ebner, J. Biol. Chem. 241 (1966) 762.
U.Brodbeck, W.L.Denton, N.Tanashi and K.E.Ebner, J. Biol. Chem. 242 (1967) 1391.
[7] K.Brew, T.C.Vanaman and R.L.Hill, Proc. Natl. Acad. Sci. U.S. 59 (1968) 491.
[8] R.G.Coffey and F.J.Reithel, Biochem. J. 109 (1968) 169.
B.Fleischer, S.Fleischer and H.Ozawa, J. Cell Biol. 43 (1969) 59.
[9] K.Brew Nature 223 (1969) 671.
[10] A.R.Williamson, in: Eassays in biochemistry, Vol. 5 (1969) p. 139.
[11] B.A.Askonas and A.R.Williamson, Biochem. J. 109 (1968) 637.
[12] G.Blobel and V.R.Potter, J. Mol. Biol. 26 (1967) 293.
[13] P.N.Campbell, O.Greengard and B.A.Kernot, Biochem. J. 74 (1960) 107.
A.von der Decken and P.N.Campbell, Biochem. J. 84 (1962) 449.
[14] G.Schreiber, H.M.Rotermund, H.Maeno, K.Weigand and R.Lesch, European J. Biochem. 10 (1969) 355.
[15] C.M.Redman, J. Biol. Chem., 244 (1969) 4308.
[16] S.J.Hicks, J.W.Drysdale and H.N.Munro, Science 184 (1969) 584.
[17] M.Takagi and K.Ogata, Biochem. Biophys. Res. Commun. 33 (1968) 55.
[18] M.Takagi, T.Tanaka and K.Ogata, J. Biochem. Tokyo 65 (1969) 651.
[19] T.Hallinan, C.N.Murty and J.H.Grant, Life Sci. 7 (1968) 225.
T.Hallinan, C.N.Murty and J.H.Grant, Arch. Biochem. Biophys. 125 (1968) 715.
[20] G.Ragnotti, G.R.Lawford and P.N.Campbell, Biochem. J. 112 (1969) 139.
[21] I.M.Arias, D.Doyle and R.T.Schimke, J. Biol. Chem. 244 (1969) 3303.
[22] Y.Kuriyama, T.Omura, P.Siekevitz and G.E.Palade, J. Biol. Chem. 244 (1969) 2017.