Comparison of the Abilities of Free and Membrane-Bound Polyribosomes Isolated from Exponential-Phase Cells of *Bacillus amyloliquefaciens* to Incorporate Amino Acids into Protein

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Schlessinger (1963) studied *Bacillus megaterium* and, on lysing cells in the presence of 10mM-tris-HCl buffer, pH 7.5, containing 10mM-Mg²⁺, 10mM-NaCl and 10mM- β -mercaptoethanol, found 33–50% of the cellular RNA was membrane-bound and was not removed by repeatedly washing with 10mM-tris-HCl buffer, pH 7.5, containing 10mM-Mg²⁺ and 10mM- β -mercaptoethanol. He further found that isolated membranes could incorporate up to 6 times, more usually 2.5–3 times, the amount of amino acids into protein per unit weight of RNA than could free ribosomes.

Moore & Umbreit (1965) studied *Streptococcus faecalis* and prepared extracts of lysozyme-pre-treated cells, suspended in a solution containing 10mM-Mg²⁺, 120mM-KCl and 10mM- β -mercaptoethanol, by passing them through a French pressure cell. It was found that 11.6% of the particulate RNA was in the membrane fraction of the lysate and these membrane-bound ribosomes were 3–5 times as effective as cytoplasmic ribosomes in the incorporation of amino acids.

In view of the importance of these conclusions, and because more refined techniques are now available for the preparation of cell extracts and knowledge of the nature of polyribosomes and the ionic conditions that affect their structure and activity has greatly increased, a reappraisal under more rigorously controlled ionic conditions seemed appropriate.

Coleman (1969*a,b,c*) studied the effect of Mg²⁺ and K⁺ on the activity of polyribosomes in *Bacillus amyloliquefaciens* and the influence of these ions on the distribution of ribosomal components between the soluble and membrane fractions of a cell lysate. This provides a suitable system for a study of the amino acid-incorporating abilities of free and bound polyribosomes and a comparison of their activities in the same organism under the different ionic conditions used by Schlessinger (1963) and Moore & Umbreit (1965).

Exponential-phase cell preparations of *B. amyloliquefaciens* were lysed with lysozyme in 50mM-tris-HCl buffer, pH 7.6, containing 10mM-Mg²⁺ and either 10mM- or 100mM-KCl (Coleman, 1969*c*). The lysates were centrifuged and the supernatant fractions retained as sources of free ribosomal material. The insoluble membrane pellets were washed with lysing buffer until no more 260nm-

absorbing material was eluted; they were then resuspended to the original volume in the same buffer. The time-course of ¹⁴C-labelled L-amino acid incorporation by each of the preparations obtained was then followed, under conditions where the Mg²⁺ and K⁺ concentrations remained unchanged, and the plateau level of incorporation determined in each case.

Table 1 shows the specific radioactivities, ¹⁴C-labelled L-amino acid incorporated into protein per unit weight of RNA, of the various preparations; the results are the mean values determined in two separate experiments. The membrane-bound polyribosomes prepared and studied in the presence of 10mM-K⁺ had a specific radioactivity 1.5–2 times that of the corresponding free polyribosome preparation. However, on study of incorporation when the K⁺ concentration of the soluble preparation had been increased to 100mM the specific radioactivity was almost doubled, in agreement with previous work of Coleman (1969*a*), to a value corresponding approximately to that of the membrane-bound material at the suboptimum K⁺ concentration of 10mM. On adjustment of the K⁺ concentration of the washed membrane suspension at 10mM to 100mM most of the ribosomal material was released (Coleman, 1969*c*) and the specific radioactivity of the released material, from which the membrane had been separated by centrifuging, was not greatly different from what it had been when membrane-bound at a suboptimum K⁺ concentration.

No difference was observed between the specific radioactivities of free or membrane-bound material prepared and studied at the optimum K⁺ concentration of 100mM, where membrane-bound material was about one-fifth of that membrane-bound at 10mM-K⁺. Further, these specific radioactivities corresponded closely to those of material prepared at 10mM-K⁺ and subsequently adjusted to 100mM-K⁺ and also to the membrane-bound material prepared and studied at 10mM-K⁺; thus under the conditions employed the specific radioactivity of the membrane-bound polyribosomes was independent of K⁺ concentration.

The above results show that *in vitro* membrane-bound polyribosomes can be more active than free polyribosomes, but only under particular conditions, and they were never more active than free material under optimum conditions for cell-free amino acid

Table 1. *Comparison of specific radioactivities of free and membrane-bound polyribosomes at different K⁺ concentrations*

¹⁴C-labelled L-amino acid incorporation experiments were carried out as described by Coleman (1959a), the only difference being that the potassium ATP and potassium phosphoenolpyruvate concentrations were halved to enable a final K⁺ concentration of 10mM to be achieved where necessary. A 60 min. incubation period at 30° was employed, during which time radioactivity incorporated into protein reached a plateau level. RNA contents of the cell fractions were determined by the orcinol method (Schneider, 1957).

Polyribosome preparation	Concn. of K ⁺ (mM)		Specific radioactivity of polyribosomes (c.p.m./mg. of RNA)
	During cell lysis	During ¹⁴ C-labelled L-amino acid incorporation	
Free	10	10	28400
Membrane-bound	10	10	49200
Free	10	100	47000
Released from membrane	10	100	54300
Free	100	100	49300
Membrane-bound	100	100	50400

incorporation. Thus the binding of ribosomes to membranes does not intrinsically enhance their cell-free protein-synthesizing ability. These findings are not necessarily inconsistent with those of previous workers, as the differences may be merely in the interpretation of results.

Thus Schlessinger (1963) introduced the observation that the protein-synthetic capacity of ribosomal material released from membranes by lowering Mg²⁺ concentration was less than when membrane-bound as evidence in favour of the greater efficiency of membrane-bound ribosomes. However, this may be a case of lowering Mg²⁺ concentration adversely affecting the activity of polyribosomes (Coleman, 1969b), an idea supported by the fact that whereas Schlessinger (1963) prepared washed membranes in the presence of 10mM-Na⁺ he placed them in a cell-free protein-synthesizing system containing a univalent cation concentration of approx. 65mM, which would be expected to result in the liberation of most of the bound ribosomes (Coleman, 1969c). Schlessinger (1963) also showed that his method of preparation of cell extracts, unlike that used in the present study, resulted in the membrane-bound ribosomal material having a higher proportion of polyribosomes than the soluble fraction.

Moore & Umbreit (1965) compared incorporating abilities of membrane-bound and soluble ribosomal material under conditions that would not result in the liberation of ribosomes from the membrane. Although their method of preparation of cell

extracts was such that ribosomes of decreased amino acid-incorporating ability might be expected (Fan, Higa & Levinthal, 1964; Coleman, 1969b), it is not possible to correlate their incorporation data with the nature of the ribosomal material in their two fractions owing to lack of resolution of components in their sucrose-density-gradient profiles. However, Moore & Umbreit (1965) account for their results in terms of a proportionality between extent of amino acid incorporation into protein and the phospholipid content of the system.

A hypothesis to explain the greater activity of the membrane-bound ribosomes at suboptimum K⁺ concentrations, consistent with the conclusion of Moore & Umbreit (1965), is that the membrane exerts a non-specific ionic effect that can be simulated by a high K⁺ concentration. The two effects may be reconciled in terms of a high concentration of K⁺ competing with Mg²⁺ for negatively charged binding sites on the ribosome, messenger and transfer RNA preventing the formation of an excess of Mg²⁺ bridges that it is suggested are inhibitory to amino acid incorporation (Coleman, 1969a). In the presence of a low K⁺ concentration, insufficient to overcome the effect of the intraribosomal Mg²⁺ bridge formation, the membrane, which also presents a series of negatively charged sites, may compete with the intraribosomal binding sites for Mg²⁺ with the formation of membrane-ribosome Mg²⁺ bridges, to such an extent that the components for protein synthesis are no longer bound with excessive firmness to the ribosomes and can thus function with optimum efficiency.

It must be emphasized that, although this work does not exclude the possibility that polyribosomes may be membrane-bound in bacterial cells for a valid physiological reason, in the absence of details of the nature of the intracellular environment the results of the studies *in vitro* discussed above do not constitute very convincing evidence that bacterial ribosomes function better *in vivo* when membrane-bound (Hendler, 1968).

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