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Review–Hypothesis

Participation of acid phospholipids in protein translocation across the bacterial cytoplasmic membrane

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Recent observations confirm the participation of acid phospholipids in protein translocation. The hypothesis proposed coupled protein translocation with transmembrane movement of acid phospholipids, their metabolism as a precursor of cell envelope components and recycling. These factors ensure the unidirectional vector value of the secretion, restoration of the membrane site competent for protein translocation and its self-organization.

Bacterial membrane; Protein translocation; Phospholipid metabolism

1. INTRODUCTION

Significant progress has been made in studying the molecular basis of exoprotein secretion and membrane protein assembly. However, some important aspects of this problem are still obscure. These are the mechanisms of thermodynamically unfavourable translocation of hydrophilic proteins across the hydrophobic bilayer membranes and its energetics. To understand these mechanisms, it is necessary to take into account the dynamic features of the membrane components, primarily phospholipids, in protein secretion.

Investigations on the role of membranes in the bacterial protein secretion have revealed a link between this process and the composition, physico-chemical state and metabolism of acid phospholipids [1]. Besides, analysis of the dynamic properties of the membranes showed the conditions necessary to maintain the turnover and transbilayer movement of their phospholipids to be the same as those required for promoting protein secretion. It suggested coupling of these transmembrane processes [1]. The most recent observations in bacteria confirm the involvement of acid phospholipids in protein translocation.

We present a short review of these data and a new model of bacterial protein secretion which suggests its coupling not only with biosynthesis and translocation of acid phospholipids but also with their metabolism as precursors of the cell envelope components and recycling. We propose a new possible explanation of the role of ATP and $\Delta\tilde{\mu}H^+$ in protein translocation. We believe them to be the factors which determine the metabolism and physico-chemical state of acid phospholipids competent for secretion.

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2. INTERACTION OF THE SECRETORY PROTEINS WITH MEMBRANE ACID PHOSPHOLIPIDS AND THEIR COUPLED TRANSLOCATION

Most secreted proteins are synthesized as precursors with an N-terminal extension (signal peptide (SP)) found obligatory and sufficient for initiating protein secretion and translocation across the membrane [2,3]. The SP structure comprises a hydrophilic basic N-terminal region followed by a stretch of apolar, mainly hydrophobic, residues forming an α-helix or β-sheet. This structure seems ideally fit for the non-specific interaction with the negatively charged surface and hydrophobic membrane interior rather than with the specific protein receptors [3].

The membrane components that are the most competent for this interaction are negatively charged acid phospholipids: phosphatidylglycerol (PG) and cardiolipin (CL). We showed PG to be less accessible to the action of phospholipases A_2 and C in cells secreting alkaline phosphatase (6%) compared to non-secreting cells (46%) [4]. Then we found PG in the membrane site interacting with the translational complex [5]. It was hydrolyzed only after the specific removal of ribosomes from the isolated cytoplasmic membranes of the secreting cells by incubating membranes with GTP, elongation factor G and puromycin. Interaction of the signal peptide of bacterial [6,7] and mitochondrial [8,9]

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metabolism into MDO can be the motive force of the phospholipid transfer and determine the unidirection of protein secretion as well as couple secretion with biogenesis of the cell envelope.

The above-mentioned lipid cycle also restores the initial molecular type of CL expended for the secretory act and competent for interaction with protein. As a result, conditions are created for supporting the secretory process. Besides, coupling of secretion with the phospholipid cycle provides the self-organization of secretion as well as the possibility of its regulation at the membrane level. PA and DAG resulting from CL and PG metabolism (fig.2) are also important for secretion. They promote the adoption of a non-bilayer configuration by phospholipids [35,36] and are the substrates of the phospholipid resynthesis [37].

In view of the above we propose a new explanation for the role of ATP and $\Delta\tilde{\mu}H^+$ in protein translocation. These energy factors were shown [38–41] to be necessary, but do not form direct sources of energy for protein translocation. Their function is considered to include the maintenance of the competent state both of the secreted protein [40,42,43] and secretory machinery [39,40]. We believe phospholipids to play an important role as intermediates in the maintenance of this competent state with ATP and $\Delta\tilde{\mu}H^+$. ATP provides the level of phospholipid metabolism necessary for secretion. It functions as the effector of phospholipase D converting CL to PG and initiates the secretion and probably, SP unfolding. On the other hand, ATP hydrolysis is required for the DG-kinase reaction of the phospholipid cycle [34] restoring the membrane site competent for secretion.

It is known that a number of proteins – soluble and membrane – are necessary for protein secretion in bacteria [44]. It can be presumed that at least some of them might be enzymes which catalyze the reactions of the phospholipid metabolism important for secretion. $\Delta\tilde{\mu}H^+$ also supports the competent state of the membrane. It provides the maximal negative charge of phospholipids, specifically by the dissociation of cardiolipin Mg salts (recently shown to exist [45]) which requires the cytoplasmic alkalization. Thus, the phospholipids can be said to participate in the transformation of membrane energy into the protein translocation. We do not rule out the possibility that protein translocation across the eukaryotic membranes is similar to that in bacteria. The necessity of phosphatidylinositol resynthesis during stimulation of secretion in pancreas [27] and the significant role of phospholipids in protein import into mitochondria [44] confirm this assumption.

4. CONCLUSIONS

Lipid-protein interaction and phospholipid metabolism play an important role in the molecular mechanism of protein translocation across the membrane.

The role of phospholipids is as follows: (i) to form the membrane site competent for protein insertion; (ii) to form the translocation site (due to flip-flop of the phospholipids and their metabolism); (iii) to provide the unidirectional vector value of the process; (iv) to restore the membrane site competent for protein translocation; (v) to regulate this process and, thus, the whole cell metabolism; (vi) to use the membrane energy for secretion. The membrane rearrangement, both structural (phospholipid translocation and formation of the non-bilayer hexagonal phase) and metabolic (interconversion as well as exchange of phospholipids and their involvement in the metabolism of envelope components), makes the translocation of the hydrophilic secreted protein thermodynamically possible.

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