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Abstracts

MO 1

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Components and Mechanisms Involved in Protein Translocation across the ER Membrane

The process of protein translocation can be divided into two phases: 1. an initiation or targeting process, which generally involves the function of the signal recognition particle (SRP) and of its receptor, and 2. the actual transfer of the polypeptide across the membrane which is only poorly understood. We have used chemical crosslinking to identify membrane proteins which are adjacent to nascent polypeptide chains as they pass through the membrane. A combination of crosslinking and reconstitution methods led to the identification of a multi-spanning membrane protein, the "translocating chain associating membrane (TRAM)" protein[1]. The TRAM protein is in vicinity of polypeptides early during their membrane transfer and it is stimulatory or required for the translocation of different secretory proteins. The main component of a putative protein-conducting channel, however, seems to be Sec61p, a membrane protein originally discovered in *Saccharomyces cerevisiae* by genetic methods. Sec61p is in proximity of translocating polypeptides throughout their membrane transfer both in yeast[2,3] and in mammals[4]. The mammalian Sec61p is highly homologous to the protein of yeast. Both have a significant sequence homology to SecYp of bacteria, indicating that the mechanism of translocation is basically the same in all organisms. In mammals, Sec61p is tightly bound to membrane-bound ribosomes, suggesting that the nascent chain is transferred directly from the ribosome into a protein-conducting channel in the ER membrane. Sec61p could be purified in a functional state as a complex containing two smaller polypeptide chains (14 and 8 kDa). The Sec61p-complex is essential for protein translocation in a reconstituted system.

1 Görlich, D., Hartmann, E., Prehn, S. & Rapoport, T.A. (1992) *Nature (London)* **357**, 47–52.
2 Sanders, S.L., Whitfield, K.L.M., Vogel, J.P., Rose, M.D. & Schekman, R.W. (1992) *Cell* **69**, 353–366.
3 Müsch, A., Wiedmann, M. & Rapoport, T.A. (1992) *Cell* **69**, 343–352.
4 Görlich, D., Prehn, S., Hartmann, E., Kalies, K.-U. & Rapoport, T.A., submitted for publication.

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MO 2

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Protein Folding and Oligomerization in the Endoplasmic Reticulum

We are trying to define the characteristics that make the lumen of the endoplasmic reticulum (ER) a unique and exceptionally efficient folding and sorting environment for proteins to be secreted, expressed on the plasma membrane or delivered to the membranes or lumen of vacuolar organelles. Using mainly viral glycoproteins (influenza HA and VSV G protein) as model proteins, we investigate, at the molecular level, the processes of folding, oligomerization and "quality control".

Our results have shown that efficacy and rate of conformational maturation of glycoproteins in the ER determines how efficiently, how fast, and in which form proteins are secreted, expressed on the plasma membrane or delivered to other organelles. Maturation intermediates, misfolded proteins or misassembled oligomers are, as a rule, retained in the ER where they are degraded. In addition to providing a mechanism for restricting the potential damage caused by the deployment of defective proteins, this system is used by the cell to post-translationally regulate expression levels of specific proteins.

To understand these reactions in further detail, we have used pulse chase experiments in liver cells, in vitro translation in the presence of microsomes, in vitro mutagenesis, and genetic approaches. Since most proteins made in the ER depend on the formation of disulfides for proper folding, we have followed the folding process by monitoring the state of oxidation. The folding and oligomerization processes have also been analysed using monoclonal antibodies to conformation-dependent epitopes, by morphological methods, by cell fractionation and by determining the degree of sugar processing.

The results show that folding of individual glycopolypeptide chains begins on the nascent chain and continues for several minutes post-translationally. For influenza HA0, the six intrachain disulfide bonds are formed starting at the top of the molecule and proceeding in a defined order down towards the membrane. The outcome of the folding process is dependent on the presence of chaperones and folding enzymes, as well as on the redox and ionic environment, temperature, and expression level. While the folding process takes place in the rough ER, the assembly of mature oligomers and certain aspects of the quality