Journal of Bioenergetics and Biomembranes, Vol. 22, No. 3, 1990

MINI-REVIEW

Protein Export in Bacteria: An Overview

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Most cells transport poteins into or across membranes. In animal cells a special role for the ribosomes bound to the endoplasmic reticulum was recognized early by Palade and coworkers who observed a parallel between the abundance of such ribosomes and the secretion of proteins, suggesting that the secreted proteins pass through the membrane as growing chains. Subsequent work by several laboratories has established the existence of a hydrophobic signal sequence which is cleaved during the transport across the membranes, and the "signal hypothesis" has been spelled out in some detail as a stimulating working model (Blobel and Dobberstein, 1975). The findings that the growth of a nascent chain is arrested by a multiple-subunit signal recognition particle and is resumed upon interacting with a membrane receptor (docking protein) provide a mechanistic basis for the coupled cotranslational translocation (see Walter *et al.*, 1984).

A similar mechanism of protein secretion and export in bacteria was formulated later and is supported by the observations that some secreted proteins are synthesized as precursors on membrane-bound ribosomes. Both biochemical studies and genetic manipulations have then provided useful, unique information on the mechanisms of protein secretion and export. Thus, in contrast to the endoplasmic reticulum of eukaryotic cells, the outside of the bacterial membrane is readily accessible to manipulation, and the use of extracellular labeling and protease digestion of protruding secreted peptides that are still attached to polysomes provides direct demonstration of cotranslational secretion. Biochemical studies by Randall have shown that the processing of signal peptides could be either co- or post-translational, and that large domains of polypeptides could be synthesized before translocation across membranes (Randall *et al.*, 1987), lending support to the notion of

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post-translational translocation advocated in "Membrane trigger hypothesis" (Wickner, 1979). Indeed, such post-translational translocation is quite common in bacteria. Genetic studies by Beckwith's and by Silhavy's groups have established that the signal peptide is essential for translocation and that several gene products are involved in protein translocation. Genetic manipulations have also provided insights into the intragenic information pertinent to the targeting of specific proteins for either secretion or membrane localization, and structural requiements for the processing of precursor proteins. Two signal peptidases have been identified in *Escherichia coli*; signal (leader) peptidase I was discovered by Wickner and appears to be general, while signal peptidase II, described by Wu, is specific for lipoproteins. Both signal peptidases have been purified, and the genes encoding for these signal peptidases have been identified and sequenced. The development of a suitable *in vitro* translocation system in *E. coli* has also helped to define and to analyze the molecular mechanism of protein export, especially with many genetically defined mutants available. A requirement of ATP on the membranes in co-translational and post-translational protein translocation has been demonstrated and is now believed to be the universal source of energy for protein translocation across membranes in eukaryotic microsomes, yeast microsomes, chloroplasts, mitochondria, and nucleus. The maintenance of the precursor molecules in a translocation-competent conformation has also been shown to be an important aspect of the translocation process, and several cytoplasmic factors are found to form a complex with precursor molecules to maintain such a competent conformation.

The last three years have seen an explosive advance in the understanding of how proteins are translocated across *E. coli* membranes. The convergence of biochemical and genetic approaches in demonstrating the involvement of the gene products of *secA*, *secB*, and *secY* (also known as *prlA*) in protein translocation has been an important development and has underscored the advantage of microbial systems for studying a physiological process. The *secA* and *secY/prlA* gene products are postulated by genetic analysis to play central roles in the translocation. Mutations in the peripheral membrane protein SecA or the integral membrane protein *SecY/PrlA* can either cause pleiotropic defects in export or relieve the export defects of signal sequence mutations; the soluble SecB protein affects the export of a subset of proteins *in vivo*. The involvement of these proteins in protein export has recently been demonstrated in the *in vitro* translocation systems. This development has been particularly satisfying, since the previously independent biochemical and genetic approaches have identified the same components involved in the process and have thus reaffirmed the validity of each approach for further analysis, e.g., the roles of other *sec* genes. The SecY/PrlA is now believed to be the translocator, or part of it, where peptides are translocated. SecA is

involved at least in the initial transport process and is believed to be related to the ATP hydrolysis, while SecB is involved in the maintenance of the translocation-competent conformation of precursor molecules. By various combinations of biophysical, biochemical, and genetic studies, the roles of signal peptides, lipids, structural aspects of mature region, precursor conformation, soluble factors, membrane components, and energetic requirement in protein translocation across membranes have begun to be understood in greater detail and should become much more defined in the near future. The technique of protein fusion has also become a powerful genetic tool to probe the membrane topology and protein export signal (Manoil and Beckwith, 1985).

This series aims to assemble articles in a single issue describing recent studies on the structure, function, and molecular genetics of components involved in the bacterial protein export that contribute to the rapid advances in the understanding of the molecular mechanisms of the process, since the similar collection in 1986 (Wu and Tai). Contributors are requested to review the past approaches and the current knowledge, and *especially encouraged* to speculate on future directions with working models, in the spirit of stimulating discussions and formulating ideas to prove, to refine, or to discard the hypotheses. Due to the nature of this collection, there are inevitable overlaps and disagreements among the authors. The series "Mini-reviews" starts fittingly with an article on the signal peptides, emphasizing the biophysical studies on the functional and mutant signal peptides and their interaction with phospholipids (Jones *et al.*), followed by an article on the signal sequence mutants that affect the proper localization (Gennity *et al.*) and by another on the enzymes involved in signal peptide cleavage and degradation (Dev and Ray). The second part consists of the genetic analysis of the components involved in the translocation process, starting with an article on general approaches (Bieker *et al.*), followed by three on the well-characterized *secA* (Oliver *et al.*), *secB* (Kumamoto), and *secY* (Ito). The third part includes two articles on the *in vitro* translocation systems (Fandl and Tai, and Mizushima and Tokuda). The fourth part includes three articles on the structural and intragenetic information on the export and sorting of some model proteins which have been studied extensively for genetic and biochemical analysis: the periplasmic maltose binding protein (Bassford), the outer membrane OmpA protein (Freudl *et al.*) and lipoproteins (Hayashi and Wu). The articles by Bassford and by Kumamoto also cover the topics on translocation-competent conformation of the precursors and protein factors that are involved in maintaining it. All of the above deal mainly with the transport of proteins across cytoplasmic membrane and the localization in the periplasm or outer membrane in *Escherichia coli.* However, proteins are rarely

exported into extracellular fluids (excretion) by *E. coli*, and in cases where it occurs in Gram-negative bacteria, special mechanisms and accessory proteins are required. This is discussed in the last chapter (Holland *et al.*).

This series has concentrated on protein export in bacteria (mainly in *E. coli*). A subsequent series will deal with the protein transport in eukaryotic microsomal membranes and organelles. A comprehensive review article on the protein transport in bacteria, in comparison to eukaryotes as well as organelles, has appeared (Saier *et al.*, 1989).

Acknowledgments

I thank all the contributing authors, Dr. Rao Sanadi, the Editor-in-Chief, and Ms. Angela DiPerri of the editorial office for making this volume possible.

References

Blobel, G., and Dobberstein, B. (1975). *J. Cell Biol.* **67**, 835–851.
Manoil, C. and Beckwith, J. (1985). *Proc. Natl. Acad. Sci. USA* **82**, 8129–8133.
Randall, L., Hardy, S. J. S., and Thom. J. (1987). *Annu. Rev. Microbiol.* **41**, 507–541.
Saier, M. H., Werner, P. K., and Muller, M. (1989). *Microbiol. Rev.* **53**, 333–366.
Walter, P., Gilmore, R., and Blobel, G. (1984). *Cell* **38**, 5–8.
Wickner, W. (1979). *Annu. Rev. Biochem.* **48**, 23–45.
Wu, H., and Tai, P. C. (1986) (editors). Protein secretion and export in bacteria. *Current Topics in Microbiology and Immunology*, Vol. 125, Springer-Verlag.