METHODS IN MOLECULAR BIOLOGY™

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Protein Secretion

Methods and Protocols

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Preface

The elucidation of the complete information content in hundreds of genomes has brought with it a surprising realization. More than a third of all the proteins in any given proteome are comprised of non-cytoplasmic polypeptides. These can be resident membrane proteins such as channels and receptors or secretory proteins such as hydrolytic enzymes and toxins. Membrane biogenesis and protein trafficking and secretion are central to the biology and pathology of the cell. Optimal protein trafficking is essential for cell viability, communication, and programmed death, for cells to modulate and yield metabolic goods from their environment, for pathogens to attack, and for hosts to fend them off.

Since all polypeptides in prokaryotes and most in eukaryotes are synthesized by cytoplasmic ribosomes, the cell has acquired tools that enable it to accurately and efficiently sort exported proteins from the cytoplasmic residents. Various specialized chaperones, pilots, and ushers have evolved to correctly recognize secretory and membrane proteins and in several instances this recognition prevents or stalls folding reactions. Moreover, this chaperone-mediated "face-control" effectively sorts extra-cytoplasmic from cytoplasmic proteins and then delivers them to complex membrane-associated cellular nanomachines. These catalyze the transmembrane crossing of the targeted polypeptides. Exported proteins come in different functional and structural flavors and are destined for residency of different subcellular compartments or the outside world. Some of them are even savvy enough to cross several prokaryotic and eukaryotic membranes before they reach their final destination. Hence we are now aware that various secretory proteins carry different export signals that act as specific address tags. In many instances, the features of these export signals are well understood and have hence predictive value when a new genome is deciphered through the use of biocomputing.

The study of protein secretion comes with some challenging biochemistry since a large part of the reactions take place at or in the membrane. Elegant genetic and biochemical approaches have been combined over the past 30 years in order to dissect the ways by which the membranes are negotiated so that the exported protein lands on the other side. Overexpression systems have allowed the purification of the subunits involved in large amounts, and this in turn facilitated structural studies. Such is the progress in this approach that, in many of the newer secretion systems, the structures of the components precede the description of biochemical functions. This is less true for the structural elucidation of membrane proteins. Despite recent progress, <200 membrane protein structures are known. In recent years, other powerful cell biology tools that can even offer real-time observation of the secretion process have become available. Finally, organism-wide proteomics is providing insight into how protein secretion is incorporated in the whole metabolic reaction network of the cell and in many instances has revealed interesting links with the rest of the cell's physiology.

The purpose of *Protein Secretion: Methods and Protocols* is to provide some examples of the multiplicity of tools that have been developed to study protein sorting, membrane targeting, transmembrane crossing, and secretion across multiple membranes. A wide variety of methods are covered that range from bioinformatics and proteomics to fundamental

enzymology and genetics to cell biology, structural analyses, and biophysics. This only reflects the highly multidisciplinary nature one expects from a mature field. It is hoped that the study of the various systems and the tools developed to decipher their secrets will provide users with inspiration in finding ways to tackle problems encountered in their research. The multiplicity of protein export systems discovered to date suggests that we are nowhere near a complete inventory. I chose to focus on well-characterized paradigms so that the reader can benefit from robust, well-established protocols in which many of the experimental wrinkles have been ironed out. Several systems have been chosen from both prokaryotic and eukaryotic organisms. The book is aimed at the biochemist, geneticist, or biologist (cell, molecular, or structural) who is a protein secretion novice and also at seasoned protein secretion experts who wish to incorporate new experimental tools in their studies. The book is also aimed at researchers who want to explore the immense biotechnological potential of secretion systems in the manipulation of protein export pathways for the production of heterologous proteins (be they biopharmaceuticals or industrial enzymes) as well as their use to develop vaccines and anti-microbials. The reader may gain insight from the difficulties encountered in the more established systems and use this rationally in the dissection of less characterized protein secretion machines, in less characterized organisms, or other cell biology and membrane-related questions.

I would like to thank the authors who have contributed to this work for their enthusiastic response and efforts; to John Walker, the series editor, for his constant help, encouragement, and vigilant eye; to Georgia Houlaki for her expert secretarial help and exceptional organizational skills; and to the staff at Humana Press who helped produce this volume.

Tassos Economou

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