Chapter 8

Probing Inner Membrane Protein Topology by Proteolysis

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Abstract

Inner membrane proteins are inserted into the membrane via α-helices. These helices do not only constitute membrane anchors but may mediate specific interactions with membrane protein partners or participate in energetic processes. The number, location, and orientation of these helices is referred to as *topology*. Bitopic membrane proteins that consist of a single membrane-embedded domain connecting two soluble domains are distinguished from polytopic ones that consist of multiple membrane-spanning helices connected by extramembrane domains. Defining inner membrane protein topology could be achieved by different methods. Here we describe a protease accessibility assay that makes it possible to define topology based on digestion profiles.

Key words Membrane protein, Inner membrane, Insertion, Topology, Transmembrane segment, Bitopic, Polytopic, Proteolysis, Protease, Proteinase K, Carboxypeptidase Y

1 Introduction

Bacterial secretion systems are multiprotein machines that catalyze the traffic of protein substrates across the cell envelope [1]. Most secretion systems described so far assemble large channels composed of inner and outer membrane proteins [1]. In secretion systems, inner membrane proteins are crucial for the assembly of platforms for pilus polymerization, substrate recruitment and selection, or energetic purposes [1]. Defining inner membrane protein topology, a term referring to the number, position, and orientation of transmembrane helices (TMHs), is therefore an important step to characterize these proteins. Depending on the number and position of these TMHs, inner membrane proteins are categorized into bitopic and polytopic proteins (Fig. 1). Bitopic membrane proteins consist of a single membrane-embedded domain connecting two soluble domains located in two different compartments. The TMH of bitopic proteins could be located at the N- or C-terminus. By contrast, polytopic membrane proteins consist of multiple TMHs connected by extramembrane domains called *loops*. Bitopic proteins

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