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Review

Membrane topology of transmembrane proteins; determinants and experimental tools

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ABSTRACT

The membrane topology refers to a two-dimensional structural information of a membrane protein that indicates the number of transmembrane (TM) segments and the orientation of soluble domains relative to the plane of the membrane. Since membrane proteins are co-translationally translocated across and inserted into the membrane, the TM segments orient themselves properly in an early stage of membrane protein biogenesis. Each membrane protein must contain some topogenic signals, but the translocation components and the membrane environment also influence the membrane topology of proteins. We discuss the factors that affect membrane protein orientation and have listed available experimental tools that can be used in determining membrane protein topology.

Contents

Abbreviations: GFP, green fluorescent protein; ER, endoplasmic reticulum; TM, transmembrane; TEV protease, tobacco etch virus protease.

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1. Introduction

The majority of membrane proteins are estimated to be alpha helical bundle types which contain at least one transmembrane (TM) domain that traverses the membrane. Soluble domains of a membrane protein thus reside on one or the other side of the membrane. Membrane topology refers to where the soluble domains are oriented relative to the plane of the membrane and how many TM domains are there in the membrane, and it offers the guidance to structure and function studies of membrane proteins.

In referring to membrane protein topology, "in" indicates cytoplasm, originated from bacteria, and "out" indicates non-cytoplasmic side; periplasm in prokaryotes and luminal side of the endoplasmic reticulum (ER), Golgi, endosomes, lysosomes or extracelluar matrix side of the plasma membrane in eukaryotes.

In this article, we review the determinants of membrane topology, protein components and membrane environment that influence membrane orientation of proteins, the global view on membrane topology of polytopic membrane proteins, and summarize currently available experimental tools that are used in determining membrane topology of proteins.

2. Membrane topology determinants

2.1. Positive inside rule

It has been observed that positively charged residues in flanking loops of TM domains in membrane proteins are predominantly found in the cytoplasmic side of the membrane, and this phenomenon is so-called the "positive inside rule" [1–3]. For bacterial membrane proteins, positive inside rule is well preserved, so that it is rare to find positively charged residues in the soluble loops facing the periplasm [1]. For membrane proteins destined to the secretory pathway in the eukaryotic cell, the soluble domain that contains the net positive sum of charged flanking residues of the TM segment is oriented to the cytoplasmic side [4,5].

Some explanations for the positive inside rule are as follows (Fig. 1). Positively charged residues in a nascent chain seem to be less translocatable across the membrane and are left in the side of the membrane where protein translation has occurred (*cis* side). Since membrane proteins are co-translationally translocated and membrane inserted, positively charged residues exposed to the *cis* side of the membrane during translation/translocation may interact with negatively charged lipid head groups and are not translocated to the *trans* side. It also could be that the translocation machinery is less accommodating for translocation of positively charged residues. Lastly, the negative membrane potential in the cytoplasmic side of the membrane may interact with positively charged residues and/or the positive membrane potential in the periplasmic side repulses positively charged residues, thus prevent translocation of positively charged residues across the membrane.

The reason that bacterial membrane proteins exhibit stronger positive inside rule compared to the membrane proteins destined to the secretory pathway in the eukaryotic cell may attribute to the membrane potential differences in the two membranes.

Interestingly, while all membrane proteins show the positive inside rule, it has been observed that nuclear-encoded mitochondrial inner membrane proteins does not exhibit noticeable positive inside rule, meaning that positively charged residues are equally found in the intermembrane space and the matrix [6]. These proteins are translocated from the cytosol, and the intermembrane space (*cis* side), thus positively charged residues may be left in the intermembrane space by interacting with lipid head groups. But, since the inner membrane potential is negative in the matrix side, electrophoretic force may facilitate translocation of some positively charged residues across the inner membrane. Of note, the distribution of negatively charged Glu residues is found over-presented in the intermembrane space side for nuclear-encoded mitochondrial inner membrane proteins [6].

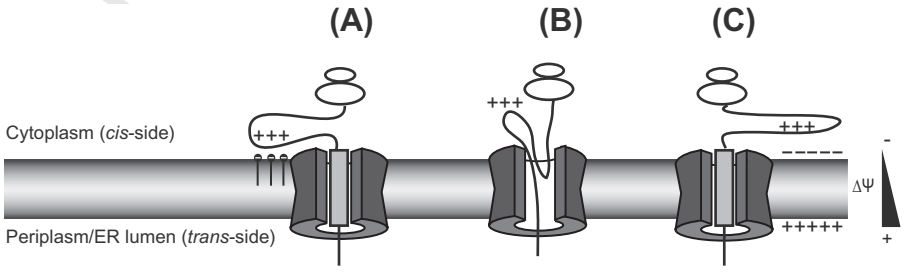

Fig. 1. Some explanations for the positive inside rule. During co-translational translocation and membrane insertion from the *cis* side of the membrane, the exposed positively charged residues of a nascent chain may interact with negatively charged phospholipid head groups (A) or the translocation machinery may disfavor translocation of positively charged residues (B). The negative membrane potential in the cytosolic side of the membrane may also influence retaining positively charged residues of a nascent chain (C).

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