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2 Review

## 6 Membrane topology of transmembrane proteins; determinants 7 and experimental tools

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### 23 ABSTRACT

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

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

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## 74 1. Introduction

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

83 In referring to membrane protein topology, “in” indicates  
 84 cytoplasm, originated from bacteria, and “out” indicates non-cyto-  
 85 plasmic side; periplasm in prokaryotes and luminal side of the  
 86 endoplasmic reticulum (ER), Golgi, endosomes, lysosomes or extra-  
 87 cellular matrix side of the plasma membrane in eukaryotes.

88 In this article, we review the determinants of membrane  
 89 topology, protein components and membrane environment that  
 90 influence membrane orientation of proteins, the global view on  
 91 membrane topology of polytopic membrane proteins, and summa-  
 92 rize currently available experimental tools that are used in deter-  
 93 mining membrane topology of proteins.

## 94 2. Membrane topology determinants

### 95 2.1. Positive inside rule

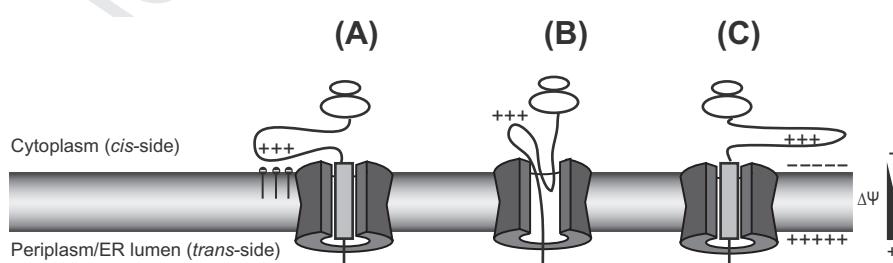
96 It has been observed that positively charged residues in flanking  
 97 loops of TM domains in membrane proteins are predominantly  
 98 found in the cytoplasmic side of the membrane, and this phenom-  
 99 enon is so-called the “positive inside rule” [1–3]. For bacterial  
 100 membrane proteins, positive inside rule is well preserved, so that  
 101 it is rare to find positively charged residues in the soluble loops fac-  
 102 ing the periplasm [1]. For membrane proteins destined to the  
 103 secretory pathway in the eukaryotic cell, the soluble domain that

contains the net positive sum of charged flanking residues of the  
 104 TM segment is oriented to the cytoplasmic side [4,5].

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

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

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



138 **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  
 139 positively charged residues of a nascent chain may interact with negatively charged phospholipid head groups (A) or the translocation machinery may disfavor translocation  
 140 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  
 141 nascent chain (C).

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