Review

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Anchors aweigh: protein localization and transport mediated by transmembrane domains

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The transmembrane domains (TMDs) of integral membrane proteins have emerged as major determinants of intracellular localization and transport in the secretory and endocytic pathways. Unlike sorting signals in cytosolic domains, TMD sorting determinants are not conserved amino acid sequences but physical properties such as the length and hydrophilicity of the transmembrane span. The underlying sorting machinery is still poorly characterized, but several mechanisms have been proposed, including TMD recognition by transmembrane sorting receptors and partitioning into membrane lipid domains. Here we review the nature of TMD sorting determinants and how they may dictate transmembrane protein localization and transport.

Transmembrane domains: not just anchors

Transmembrane proteins account for 20–30% of all proteins encoded in the genome of eukaryotic organisms [1]. They include bitopic proteins that span the membrane only once and polytopic proteins that cross the membrane multiple times. To perform their functions, transmembrane proteins must be transported and localized to the correct intracellular compartment [e.g., endoplasmic reticulum (ER), Golgi apparatus, plasma membrane, endosomes] and in many cases traffic in a regulated manner between different compartments (e.g., cycling of the transferrin receptor between the plasma membrane and endosomes, ligand-induced endocytosis of signaling receptors) (Figure 1). Intracellular localization and traffic are often determined by information contained within the cytosolic domains of the transmembrane proteins. This information generally comprises linear amino acid motifs or folded domains that interact with components of protein coats, thus leading to selective incorporation of membrane proteins into transport vesicles [2]. However, although cytosolic sorting determinants have received the most attention in the protein-trafficking field, numerous studies over the past 25 years have shown that TMDs also contribute to protein localization and transport.

TMDs usually comprise a stretch of 17–25 (average 21) hydrophobic amino acid residues [3] that are structured as an α-helix [4]. A bioinformatics analysis of integral membrane proteins encoded in eukaryotic genomes revealed a strong correlation between the intracellular localization of the proteins and the exact length and amino acid composition of their TMDs [5]. For instance, the TMDs of ER proteins were found to be shorter than those of plasma membrane proteins. Furthermore, the TMDs of plasma membrane proteins exhibit an asymmetric distribution of amino-acids along the α-helix, with more valine and glycine residues toward the exofacial side and more leucine residues toward the endofacial side of the membrane [5]. By contrast, the TMDs of ER proteins do not display such asymmetry. These findings provided a global perspective on previous experimental analyses of the contribution of TMDs to various sorting events, including localization to the ER and the Golgi apparatus, endocytosis from the plasma membrane, transport from endosomes to the *trans*-Golgi network (TGN), and entry into intraluminal vesicles (ILVs) of multivesicular bodies (MVBs) (Figure 1 and Table 1). Here we review the critical roles played by TMDs in intracellular sorting and discuss the mechanisms that have been proposed to explain TMD-mediated sorting.

Sorting events mediated by TMDs

ER retention and degradation

Retention of proteins in the ER was one of the first sorting processes found to depend on TMDs (Table 1). Studies on the assembly and transport of hetero-oligomeric membrane complexes such as the T-cell antigen receptor (TCR) (comprising eight type I transmembrane subunits, $\alpha\beta\gamma\delta\varepsilon_2\zeta_2$) showed that only fully assembled complexes reach the cell surface, whereas unassembled subunits or partial complexes are retained in the ER [6,7]. For some unassembled subunits, ER retention is followed by degradation [6,7] through a ubiquitin/proteasome-dependent process known as ER-associated degradation (ERAD) [8]. Information leading to ER retention and ERAD targeting was mapped to the TMDs of the proteins [7]. The TMDs of TCR subunits are unusual in that they contain one or two

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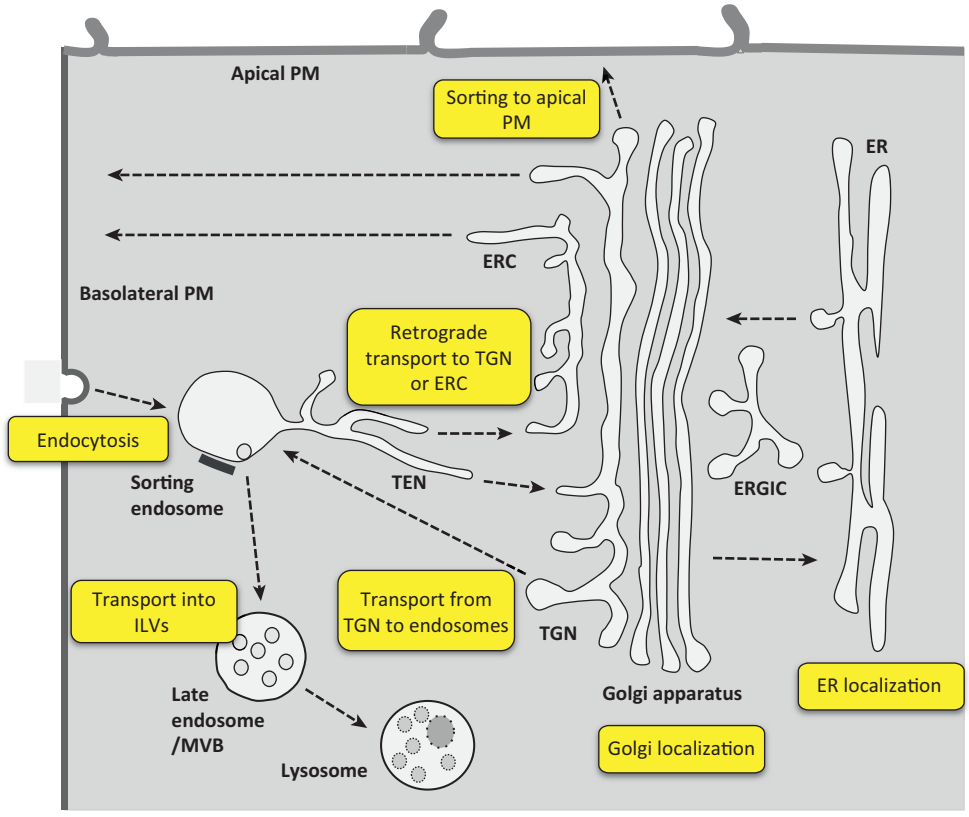

Figure 1. Sorting processes mediated by transmembrane domains (TMDs). Schematic representation of intracellular transport pathways (arrows) and processes in which TMDs participate in protein sorting (yellow boxes). Newly synthesized transmembrane proteins can be transported from the endoplasmic reticulum (ER) to the Golgi apparatus, from where they return to the ER (ER–Golgi recycling) or continue on to the *trans*-Golgi network (TGN) and the plasma membrane (PM) (secretory pathway). The ER–Golgi intermediate compartment (ERGIC) may play a role in both anterograde and retrograde transport steps. In polarized epithelial cells, the PM is specialized in apical and basolateral domains to which proteins are differentially sorted (polarized sorting). PM proteins can be internalized into endosomes (endocytosis) from where they can return to the PM via the endocytic recycling compartment (ERC) (endocytic recycling) or undergo transport to the TGN via the tubular endosomal network (TEN) (retrograde transport) or to late endosomes/multivesicular bodies (MVBs) and then to lysosomes (lysosomal transport). In MVBs, proteins can either remain in the limiting membrane or be transported into intraluminal vesicles (ILVs) (MVB pathway). Some proteins cycle between the TGN and endosomes (TGN–endosome recycling).

charged residues (basic in the TCR-α and -β subunits; acidic in the CD3-γ, -δ, -ε, and -ζ subunits) that contribute to ER retention and ERAD targeting, as demonstrated by the fact that mutating them to hydrophobic residues disrupts both processes [7]. Moreover, simple placement of a charged or strongly polar residue (i.e., asparagine, glutamine) in the TMD of a reporter plasma membrane protein can confer localization to the ER and targeting to ERAD [9]. Whether strongly polar residues cause just ER retention or additional ERAD targeting depends on the nature of the residue and its position within the TMD. These outcomes are also dependent on the length of the TMD, with shorter TMDs enhancing and longer TMDs diminishing the effects of charged residues [10]. Similar to the TCR subunits, the α-chain of the high-affinity IgE receptor contains a charged residue [11] and the membrane IgM (mIgM) subunit of the B-cell antigen receptor (BCR) [12] contains a large number of polar residues (nine serine and threonine residues) that contribute to retention of the unassembled subunits in the ER. The TMDs of these proteins also mediate subunit interactions, such that subunit assembly abrogates ER retention and ERAD targeting [13]. By coupling oligomer assembly with export from the ER, the TMD-dependent sorting machinery participates in quality control, ensuring that only fully assembled complexes reach the plasma membrane.

Similar determinants account for the localization of ER-resident proteins and some viral envelope glycoproteins to the ER (Table 1). For example, charged or hydrophilic residues in the TMDs contribute to the ER localization of the cellular proteins cytochrome P450 2C1 [14] and p24 [15], as well as envelope glycoproteins from hepatitis C virus [16] and Dengue virus [17]. The shorter length of TMDs from UBC6 (17 residues) [18] also determines ER localization. Finally, the TMD of the ER chaperone Cosmc directs ER retention, although in this case it is by virtue of a cysteine residue that participates in disulfide-bonded dimerization [19].

Golgi localization

TMDs are also a major determinant of protein localization to the Golgi apparatus, albeit often in cooperation with other topologic domains (Table 1). This role of TMDs was first demonstrated for the coronavirus E1 glycoprotein [20] and later found to apply to other viral envelope glycoproteins [17,21,22]. Additionally, TMDs contribute to Golgi localization of a large number of glycosylation enzymes that process the carbohydrate chains of newly synthesized glycoproteins and glycolipids as they traverse the Golgi apparatus [23,24]. Likewise, other Golgi proteins such as