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Review

Targeting pathways of C-tail-anchored proteins ☆

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ABSTRACT

A large group of diverse, functionally important, and differently localized transmembrane proteins, share a particular membrane topology, consisting of a cytosolic N-terminal region, followed by a transmembrane domain close to the C-terminus. The C-terminal membrane anchor of these tail-anchored (TA) proteins generally represents the sole targeting determinant, and becomes available to targeting factors only after release of the finished polypeptide from the ribosome. Hence, TA proteins do not have a chance to interact co-translationally with Signal Recognition Particle and are delivered post-translationally to all target membranes, including the ER. Recent work has demonstrated the existence of different biogenetic pathways for TA proteins. Notably, some are able to efficiently translocate their C-terminus across protein-free bilayers without the participation of any membrane or cytosolic protein, while others require assistance from cytosolic chaperones and membrane receptors. In this review, we summarize current knowledge on the different insertion pathways, with emphasis on a recently discovered chaperone system that operates in fungi as well as in higher eukaryotes to deliver TA proteins to the ER (called Guided Entry of Tail-anchored Proteins (Get) system and Transmembrane Recognition Complex (TRC), in yeast and mammals, respectively). We suggest that the final insertion step of TA proteins does not require membrane proteins, but that different competing chaperone systems ensure precise delivery to defined targets while preventing inappropriate insertion into otherwise permissive bilayers. This article is part of a Special Issue entitled Protein translocation across or insertion into membranes.

Contents

Abbreviations: b5, cytochrome b5; COE, chloroplast outer envelope; ER, Endoplasmic Reticulum; Get, Guided Entry of Tail-anchored Proteins; Hsc, Heat Shock Cognate Protein; Hsp, Heat Shock Protein; OMM, outer mitochondrial membrane; RRL, rabbit reticulocyte lysate; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; SRP, Signal Recognition Particle; Syb, synaptobrevin; TMD, transmembrane domain; TOM, translocase of the outer membrane; TRC40, Transmembrane Recognition Complex subunit of 40 kDa; Ubl, Ubiquitin like domain

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1. Introduction

Tail-anchored (TA) proteins are transmembrane polypeptides characterized by an N-terminal functional cytosolic region anchored to the lipid bilayer by a single transmembrane domain (TMD), followed by a lumenal polar sequence no longer than 30 residues [1]. Bioinformatic analyses indicate that TA proteins are well represented

in all three domains of life, where they carry out a variety of essential functions that benefit from, or require, membrane anchorage, such as membrane fusion in vesicular transport, protein translocation, regulation of apoptosis, storage of transcription factors, and enzyme catalysis [2–5].

Because the C-terminal TMD constitutes the only membrane-targeting sequence and because it emerges from the ribosome tunnel only after termination of translation, TA proteins must insert into all their target membranes—endoplasmic reticulum (ER), outer mitochondrial, outer chloroplast, peroxisomal, and prokaryotic cytoplasmic membranes—by post-translational pathways. Other, non-TA, membrane proteins of mitochondria, plastids and peroxisomes also insert by post-translational mechanisms, however, proteins targeted to the ER- and the bacterial cytoplasmic membrane generally use the co-translational pathway mediated by Signal Recognition Particle (SRP) [6]. TA proteins constitute a clear-cut exception to this rule. The unique mechanisms by which the members of this group of functionally important proteins achieve their final subcellular localization have recently attracted a great deal of interest, and constitute the subject of this review.

A number of comprehensive review articles on TA proteins have been published in the recent and less recent past [1,7–12], and the reader is referred to these papers, as well as to the publications reporting bioinformatic analyses [2–5,12], for detailed information on the localization and functions of TA proteins in different organisms. In this review, we will focus on recent progress on TA protein biogenesis, discussing unanswered questions and attempting to give a unified picture of the general mechanisms underlying their targeting to—, and integration into membranes.

As mentioned above, TA proteins can be inserted from the eukaryotic cytosol into a number of target membranes, i.e., the ER, the outer mitochondrial membrane (OMM), the Chloroplast Outer Envelope (COE), and the peroxisomal membrane. Examples of TA proteins specifically targeted to these different membranes are given in Table 1. The ER represents the major destination of TA proteins [3], and from this initial insertion site many are then exported to different compartments of the endo-membrane pathway (see Table 1). We will first discuss recent advances in our understanding of insertion mechanisms of ER-targeted TA proteins, then summarize what is known on their targeting to other organelles, and finally attempt to give a conceptual framework in which to fit our present knowledge on TA protein targeting.

2. Insertion of ER-targeted TA proteins

Although it was realized early on that TA proteins are directed to the ER only after their release from the ribosome, it was considered possible that they might translocate their C-terminus by post-translational engagement of the Sec61 translocon. However, work with a number of substrates in different systems (mammalian and yeast, the latter both *in vivo* and *in vitro*) has excluded a role for Sec61 in TA protein transmembrane integration [13–16]. It has instead become apparent that more than one Sec61-independent pathway operates in ER targeting of TA substrates. More specifically, *in vitro* studies have revealed the existence of an *unassisted* pathway by which substrates with weakly hydrophobic TMDs spontaneously integrate into the lipid bilayer, and *chaperone-mediated*, energy-requiring pathways, by which most ER-targeted TA substrates reach their destination.

2.1. Unassisted insertion of ER-targeted TA proteins

Mammalian cytochrome b5 (b5) is the most thoroughly investigated ER TA protein capable of unassisted insertion. Early studies indicated that purified b5 could associate with pure phospholipid liposomes with a hairpin topology (N and C-terminus on the outside of the vesicle; [17]). Much later, our laboratory, using stringent proteolysis protection assays for *bona fide* transmembrane integration of TA proteins, demonstrated that b5, translated *in vitro* in rabbit reticulocyte lysate (RRL) translocates its C-terminus across pure lipid bilayers as rapidly and as efficiently as across microsomal membranes [16]. This work left open the possibility that chaperones of the RRL might be required to keep b5 in an insertion competent form. To investigate this possibility, we expressed b5 as fusion protein and purified it free of chaperones. A complete lack of influence of RRL on the extent and kinetics of transmembrane integration of this protein into pure lipid vesicles demonstrated unequivocally that, at least *in vitro*, b5 can translocate its C-terminus across the lipid bilayer in the absence of any membrane or cytosolic protein, and that these do not even facilitate the insertion process [18]. Interestingly, however, the presence of even low concentrations of cholesterol in the vesicles sharply inhibits the unassisted integration process [16].

Since it was known that other TA proteins do require ER membrane protein(s) and energy for their insertion [13,19], we investigated what feature of b5 is responsible for its capacity to insert without assistance. Production of chimaeric proteins and mutagenesis experiments

Table 1
Examples of TA protein targeting.

Target membrane	TA protein	Function	Notes	References
ER	Sec61β	Protein translocation		[23,25]
	Ramp4	Protein translocation		[26]
	Synaptobrevin-2	SNARE required for synaptic vesicle exocytosis	Also known as VAMP (Vesicle Associated Membrane Protein)-2. Transported down the secretory pathway from the ER to synaptic vesicles in neuronal cells	[13]
	Sed5	Golgi SNARE (Yeast orthologue of mammalian syntaxin 5)	Transported from the ER to the Golgi complex	[29]
	Cyt b5	Lipid metabolism in the ER		[62,74]
OMM	Small TOM proteins	Protein translocation		[73]
	OMb5	Enzymatic	Mitochondrial isoform of cyt b5	[62,74]
	VAMP-1B	SNARE of unknown function	Splicing variant of ER-targeted isoform (VAMP, or synaptobrevin-1A)	[61]
	Bcl-XL, Bak	Regulation of apoptosis		[68]
COE	*A. thaliana* cyt b5 isoform At1g26340	Enzymatic		[75]
	Outer Envelope Membrane Protein 9	Unknown		[77]
	Toc 33, 34	Protein translocation		[76,77]
Peroxisomes	Pex26	Peroxisome biogenesis		[80]
Dual targeting: ER and OMM	Bcl-2	Regulation of apoptosis		[81]
Dual targeting: OMM and peroxisomes	Fis1	Mitochondrial and peroxisomal fission		[59]