JCB Mini-Review

The tale of tail-anchored proteins: coming from the cytosol and looking for a membrane

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A group of integral membrane proteins, known as C-tail anchored, is defined by the presence of a cytosolic NH_2-terminal domain that is anchored to the phospholipid bilayer by a single segment of hydrophobic amino acids close to the COOH terminus. The mode of insertion into membranes of these proteins, many of which play key roles in fundamental intracellular processes, is obligatorily posttranslational, is highly specific, and may be subject to regulatory processes that modulate the protein's function. Although recent work has elucidated structural features in the tail region that determine selection of the correct target membrane, the molecular machinery involved in interpreting this information, and in modulating tail-anchored protein localization, has not been identified yet.

Introduction

Approximately 30 years ago, the signal hypothesis provided a conceptual basis that led to the identification of a limited number of molecular machines that direct groups of proteins to shared locations within the cell (for review see Blobel, 2000). In addition, however, investigations during the past years have revealed the existence of proteins that reach their target organelle by unconventional and poorly understood mechanisms. Among these, because of the variety of their locations and functions (Table I), tail-anchored (TA)* proteins have recently received a good deal of interest.

TA proteins constitute a class of integral membrane proteins that are held in the phospholipid bilayer by a single stretch of hydrophobic amino acids close to the COOH terminus, the entire functional NH_2-terminal portion facing the cytosol. They are found on essentially all membranes abutting the cytosol, where they carry out a variety of enzymatic and regulatory roles in cellular metabolism, in protein localization, and in membrane traffic (see Table I). Two examples of TA proteins that play central roles in cell physiology are soluble *N*-ethylmaleimide–sensitive factor attachment protein receptors (SNAREs; for review see Chen and Scheller, 2001) and Bcl-2 family members (for review see Cory and Adams, 2002). In both these examples, each protein's function—targeted membrane fusion or regulation of apoptosis, respectively—is inextricably linked to its specific localization. Thus, understanding the targeting and insertion mechanisms of these proteins and the underlying regulation is an issue with wide implications for cell biology.

TA proteins lack an NH_2-terminal signal sequence, and their membrane-interacting region is so close to the COOH terminus that it emerges from the ribosome only upon termination of translation. This hydrophobic region is, therefore, unlikely to interact with signal recognition particle (SRP), which binds signal peptides or signal anchors only as long as they are part of a nascent polypeptide chain. Thus, TA proteins must reach their target membranes, including the ER, posttranslationally, and this biosynthetic route is what distinguishes them from classical type II membrane proteins (defined as proteins having a single transmembrane domain (TMD) with N-cytosolic, C-exoplasmic orientation), which are delivered to the ER by the SRP-dependent cotranslational pathway (Fig. 1). By this biosynthetic criterion then—and considering that the last ~40 amino acids of the nascent chain are sequestered within the eukaryotic large ribosomal subunit (Blobel and Sabatini, 1970)—TA proteins should be considered as such if the membrane-interacting domain is followed by no more than ~30 residues. For borderline cases, a more rigorous criterion would be based on the experimental assessment of lack of SRP interaction.

Because of the small number of polar residues downstream to the hydrophobic domain, it was initially difficult to verify whether or not TA proteins actually span the bilayer, so that their exact topology remained controversial for a long

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*Abbreviations used in this paper: MOM, mitochondrial outer membrane; SNARE, soluble *N*-ethylmaledimide–sensitive factor attachment protein receptor; SRP, signal recognition particle; TA, tail-anchored; TMD, transmembrane domain; TOM, translocase of the outer mitochondrial membrane; UBC6, ubiquitin conjugating enzyme 6.

Key words: endoplasmic reticulum; membrane traffic; mitochondrial outer membrane; protein targeting and translocation; transmembrane domain

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Table I. **Different functions and localizations of TA proteins**

Function	Examples	Localization
Enzymatic	Cytochrome b(5)	ER
	MOM isoform of cytochrome b(5)	MOM
	Heme oxygenase I and II	ER
	UBC6	ER
Protein localization		
Translocation	Sec61γ, Sec61β	ER
	TOM5, TOM6	MOM
	Pex15p	Peroxisomes
Adaptors	OMP25	MOM
Vesicular traffic		
SNARE proteins	Target SNAREs (Syntaxins) Vesicular SNAREs (e.g., Synaptobrevins)	Target membranes for vesicular fusion
Tethering proteins	Giantin	Transport vesicles Golgi complex
Regulation of apoptosis (Bcl-2 family)	Bcl-2	MOM and ER
	Bcl-X_L	MOM
	Bax	Cystol and MOM
Constituent of viral envelope	Us9 protein of α herpes viruses	Trans-Golgi network

time (Kutay et al., 1993). This difficulty was subsequently overcome by the use of recombinant TA proteins with N-glycosylation sites engineered to the extreme COOH-terminal polar region (Kutay et al., 1995; Masaki et al., 1996; Honsho et al., 1998; Pedrazzini et al., 2000). Glycosylation of these COOH-terminal tags within the ER lumen occurs in vivo as well as in cell-free translation systems to which microsomes are added after completion of polypeptide synthesis, formally demonstrating that TA proteins can translocate their COOH terminus across the bilayer and that this translocation occurs posttranslationally (Kutay et al., 1995; Pedrazzini et al., 2000).

Much of the research on TA protein biosynthesis of the past few years has highlighted the importance of the tail region in the initial targeting from the cytosol to membranes, in the subsequent trafficking within the secretory pathway and, in some cases, in directly influencing the protein's function as well. It is worth emphasizing that, because each TA protein is equipped with its own characteristic tail, this region has much more complex and diversified roles than those of the simple lipid anchors of fatty acylated and isoprenylated proteins.

Despite the recent progress, the fundamental question of the nature of the cellular machinery involved in TA protein insertion into membranes has not been answered yet. In this review, we will give a brief account of what we have learned about TA protein trafficking over the past few years, to then discuss the major unanswered questions as well as novel aspects of regulation of this class of proteins.

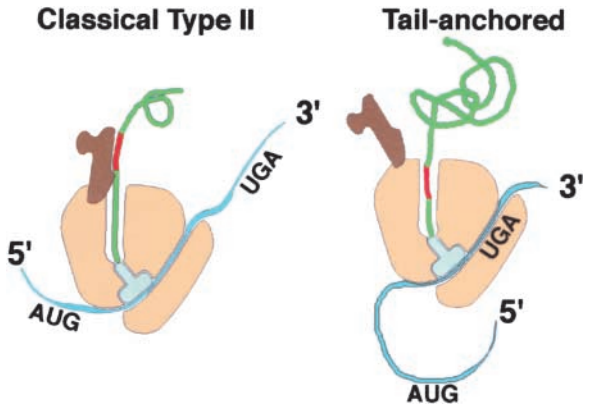

Figure 1. **Membrane insertion of tail-anchored proteins occurs after release from the ribosome.** The figure illustrates the fundamental difference between the biosynthesis of a classical type II membrane protein (left) and that of a C-tail–anchored protein (right). For type II proteins, the signal anchor (red) emerges from the ribosome before termination of translation, so that it can interact with SRP (brown). For TA proteins, the hydrophobic membrane interacting sequence (red) is still sequestered within the ribosome when the stop codon is reached, so that it never becomes available to SRP.

Tail-anchored proteins insert into a limited number of intracellular membranes and reach destinations within the secretory pathway by membrane traffic

Although results from early in vitro studies suggested that at least some TA proteins (e.g., cytochrome b[5]) could associate nonspecifically with any membrane, it is now clear that, in vivo, TA proteins insert into a limited number of intracellular membranes. Indeed, to reach compartments of the secretory pathway, TA proteins are first inserted into the ER and then delivered to their final destination by vesicular transport (Jäntti et al., 1994; Kutay et al., 1995; Linstedt et al., 1995; Pedrazzini et al., 1996). Likewise, it appears that also peroxisomal TA proteins reach the peroxisome after insertion into the ER (Elgersma et al., 1997) or into a specialized domain thereof (Mullen and Trelease, 2000). In contrast, TA proteins destined for the mitochondrial outer membrane (MOM) and the chloroplast envelope are targeted directly from the cytosol (Borgese et al., 2001; unpublished data). Thus, upon release from the ribosome, TA proteins must discriminate only between the ER and the MOM (and the plastid surface in plants).

Experiments with chimaeras between the tail of MOM-directed proteins and the cytosolic region of ER-directed proteins or soluble reporters have demonstrated that the features determining discrimination between the MOM and the ER are generally contained in the COOH-terminal tail (Nguyen

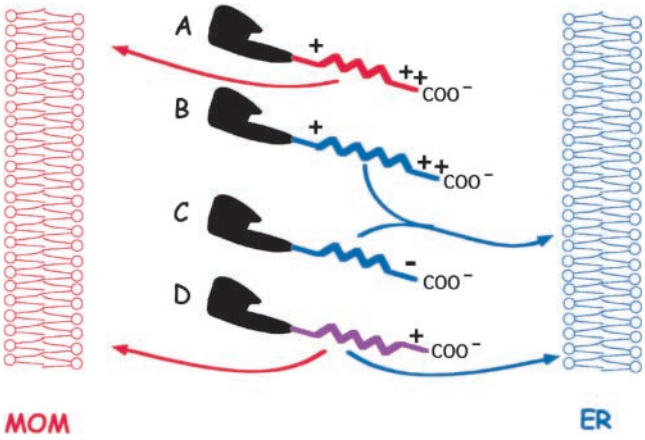

Figure 2. **Features that determine specific targeting of tail-anchored proteins to the mitochondrial outer membrane (MOM) or ER.** The cytosolic domains of the four proteins A–D are shown as a gray irregular form, whereas the the color of the COOH-terminal tail is matched to that of the corresponding target membrane. The transmembrane domain is shown as a zig-zag line. A short transmembrane domain, flanked on both sides by positively charged residues, determines targeting to the MOM (A). Loss of either of these features results in targeting to the ER (B and C). Proteins bearing tails with intermediate features (purple), i.e., a slightly lengthened hydrophobic domain and/or reduced positive charge, may be delivered to both the MOM and the ER (D). Targeting and insertion are in some cases subject to regulation (see text for details).

et al., 1993; De Silvestris et al., 1995; Isenmann et al., 1998; Nemoto and De Camilli, 1999) (Fig. 2). In MOM-targeted proteins, this region is often characterized by a rather short TMD (<20 residues), flanked by basic amino acids (Fig. 2 A). Increasing the length of the TMD or decreasing the positive charge of the flanking regions has revealed that these features are indeed important for mitochondrial targeting (Kuroda et al., 1998; Isenmann et al., 1998; Borgese et al., 2001; Motz et al., 2002; Kaufmann et al., 2003). On the other hand, there are exceptions to the short TMD rule, since some MOM-directed TA proteins have membrane anchors of considerable length and hydrophobicity (e.g., Bax, a member of the Bcl-2 family), suggesting that more subtle sequence information in this region may sometimes be involved in targeting. Finally, interactions of the cytosolic domain may in some cases play an important role, as recently reported for Bcl-2 (Shirane and Nakayma, 2003).

TA proteins without specific targeting information for the MOM are inserted into the ER, which is able to accept TMDs of different lengths and sequence followed by COOH-terminal polar regions of varying size and charge (Fig. 2 B and C). MOM-directed proteins mutated to have a longer TMD or less positive charges in the flanking regions are rerouted to the ER. Likewise, TA proteins with artificial TMDs, consisting of repeated leucines or repetitions of strings of a few hydrophobic aminoacids, are also inserted efficiently (Whitley et al., 1996; Yang et al., 1997; Honsho et al., 1998). From these observations, it appears likely that the ER is the default destination for TA proteins (but see also Kim et al., 1999).

The permissivity of the ER for TA protein insertion combined with the fact that the targeting information for the MOM is not contained in a discrete signal, but rather appears to be the result of the summation of two continuously distributed physicochemical variables (TMD length and degree of flanking positive charge), has the important consequence that proteins with weak MOM targeting features may be delivered to both the ER and the MOM (Fig. 2 D). As discussed in the last section of this review, such dual targeting may be subject to regulation with important functional implications.

Once inserted in the ER, a TA protein will either be retained there or transported to alternative final destinations. In these sorting processes, the tail region, again, plays an important role. A positively charged lumenal sequence appears to determine transport to peroxisomes (Elgersma et al., 1997; Mullen and Trelease, 2000). Sorting along the secretory pathway is instead strongly influenced by the physicochemical properties of the TMD. For a number of TA proteins, residence in the ER requires a short and moderately hydrophobic membrane anchor, which may determine both slow entry into transport vesicles and retrieval from the early Golgi (Pedrazzini et al., 2000). Lengthening the TMD of cytochrome b(5) (Pedrazzini et al., 1996), ubiquitin conjugating enzyme 6 (UBC6; Yang et al., 1997), or the ER SNARE Ufe1p (Rayner and Pelham, 1997) results in the escape of these proteins from the ER and their transport down the secretory pathway. A systematic investigation of the properties of the TMD of cytochrome b(5) required for ER residence revealed that moderate hydrophobicity, rather than length, is the crucial feature (Bulbarelli et al., 2002). TMD length or hydrophobicity may also influence further sorting of TA proteins along the secretory pathway (e.g., Reggiori et al., 2000).

In addition to simple physicochemical characteristics, sequence-specific features of the TMD may also be involved in sorting of TA proteins within the secretory pathway. An example is provided by the target SNAREs Syntaxins 3 and 4, which localize respectively to the apical and basolateral plasma membrane of polarized epithelial cells (Low et al., 1996). The hydropathy profiles of the tail region of the 2 Syntaxins are similar, yet experiments with GFP fusion proteins suggest that the TMDs do play a role in determining the polarized distribution of these TA proteins (Bulbarelli et al., 2002). Sequence-specific information in the TMD may also be important for TA protein function, as is apparently the case for SNAREs (Rohde et al., 2003).

Although the TMD plays a key role in TA protein trafficking, the cytosolic domain, in a less generalizable fashion, also often contributes to their sorting and finetunes their distribution within the secretory pathway. This appears to be the case for the Golgi-localized TA proteins studied so far (Linstedt et al., 1995; Misumi et al., 2001; Bulbarelli et al., 2002). Likewise, the determinants responsible for SNARE trafficking, which frequently involves regulated recycling between two or more compartments, reside both in the TMD and in the cytosolic domain (Joglekar et al., 2003; and references therein).

The role of the TMD in membrane protein trafficking is, of course, not limited to TA proteins (Bulbarelli et al., 2002). However, in the case of multidomain proteins, its contribution is often obscured by the presence of multi-

ple sorting determinants, located in different parts of the polypeptide. TA proteins, because of their simplicity, and because of the nearly complete absence of a lumenal domain, provide useful models for the investigation of the molecular basis of TMD-dependent sorting. The use of suitable TA reporter constructs, containing a neutral cytosolic domain devoid of sorting information, should hopefully lead to an understanding of the mechanism through which TMDs exert their effects, whether by interactions with other proteins, with bilayer lipids, or with both these classes of molecules.

What is the nature of the cellular machinery involved in TA protein targeting and insertion?

As detailed earlier, TA proteins can translocate their COOH terminus posttranslationally across the ER membrane, and presumably across the MOM and choloroplast envelope as well, although this has not been formally demonstrated so far. Thus, an obvious question is whether the classical translocation machinery—the Sec61 translocon in the ER (Rapoport et al., 1996), the translocase of the outer mitochondrial membrane (TOM; Neupert, 1997), and translocase of the outer chloroplast membrane (Jarvis and Soll, 2002) systems for mitochondria and chloroplasts, respectively—are involved.

Until recently, the possible involvement of Sec61 was addressed only in in vitro binding assays that did not follow translocation of the COOH terminus of the analyzed protein. These studies yielded contradictory results, on the one hand indicating that TA proteins could bind to Sec61-depleted mammalian (Kutay et al., 1995) or yeast microsomes (Steel et al., 2002), on the other hand revealing a weak cross-link between Sec61 and synaptobrevin at short times after binding of this TA protein to microsomes (Abell et al., 2003). In addition, it was found that synaptobrevin binding to microsomes requires at least one trypsin-sensitive component, different from Sec61 (Kutay et al., 1995). This component, however, was not required by cytochrome b(5) or Bcl-2 (Kim et al., 1997), suggesting that this or these unidentified molecules may be specifically involved in the targeting of subgroups of TA proteins.

We have recently reinvestigated the possible involvement of Sec61 by applying to the yeast system a rigorous assay for translocation, based on the in vivo utilization of an N-glycosylation consensus sequence engineered to the COOH terminus of mammalian cytochrome b(5) (Yabal et al., 2003). Yeast strains defective in components of the Sec61 translocon or in accessory proteins involved in signal peptide-driven co- or posttranslational translocation were tested for their ability to glycosylate the COOH terminus of this b(5) construct. None of the mutations or deletions had detectable effects on the in vivo translocation of b(5)'s tail, which was rapidly glycosylated under conditions in which signal peptide-driven posttranslocational translocation was abolished. These results strongly suggest that translocation of TA protein tails occurs by a mechanism not involving the Sec61 translocation machinery. This conclusion is nicely matched by the observation that Sec61 function is also not required for extraction from the membrane of another TA protein (UBC6; Walter et al., 2001), a process that leads to proteasomal degradation of ER proteins and that is generally based on the retrotranslocational activity of Sec61p (Rapoport et al., 1996). Nonetheless, the work of Yabal et al. (2003) does not formally exclude that TA protein insertion occurs through a novel function of the Sec61 translocon, not compromised by the numerous mutations analyzed in that study. In either case, the results demonstrate the involvement of a mechanism distinct from that of signal peptide–driven posttranslational translocation, and set the stage for research aimed at elucidating this mechanism.

For MOM-targeted TA proteins, in vitro experiments addressing the involvement of the TOM system have been performed using two model proteins, Bcl-2 and VAMP IB. The two proteins compete with each other for binding to the MOM, but neither of them show impaired mitochondrial association in the presence of matrix-directed preproteins (Millar and Shore, 1996; Lan et al., 2000; Motz et al., 2002). Conflicting results have been reported concerning the trypsin sensitivity of the Bcl-2 binding sites on the mitochondrial surface (Nakai et al., 1993; Motz et al., 2002), however an interaction between Bcl-2 and a glutathione *S*-transferase–TOM20 fusion protein has been reported (Schleiff et al., 1997), and a recent paper has shown that Bcl-2 binding to yeast mitochondria is partially dependent on TOM20 (Motz et al., 2002). Interestingly, mutations in other essential components of the TOM system were without effect in this assay (Motz et al., 2002). Together, the results suggest that, as in the case of the ER, the MOM insertion of TA proteins involves a novel mechanism that deserves further study.

The energy requirements for membrane insertion have also been investigated. ATP is required for the binding of synaptobrevin I to microsomes (Kutay et al., 1995), and of VAMP IB to mitochondria (Lan et al., 2000), but not for the membrane association of cytochrome b(5) or Bcl-2 (Kim et al., 1997). However, when bona fide translocation of the COOH terminus of cytochrome b(5) was assayed (by N-glycosylation of a COOH-terminal tag), a requirement for low ATP concentrations was revealed (Yabal et al., 2003). The ATP requirement for VAMP IB's insertion into the MOM has been reported to be related exclusively to chaperone function (Lan et al., 2000), and this could be the case also for the other investigated proteins. Unfortunately, the relevant chaperone(s) have not been identified so far, nor is it known whether their function is limited to preventing the aggregation of newly synthesized TA proteins, or whether they are more specifically involved in guiding TA proteins to the correct target membrane.

In conclusion, the available evidence suggests that the classical import machinery of the ER and mitochondria do not participate in the translocation of the COOH terminus of TA proteins across the lipid bilayer. The specificity of the targeting process does suggest that proteins are involved, however differences in the lipid composition of the MOM and the ER membrane (Colbeau et al., 1971) could also be important. It is also conceivable that the targeting event requires proteins, but that the subsequent translocation of the COOH terminus occurs directly across the bilayer lipids of the MOM and the ER membrane, whose lipid compositions would be permissive for this event.

It may seem surprizing that a full 10 years after many of the components of other translocation machines have been identified, there is still so much ignorance on TA protein translocation. This is partly because the realization that these proteins undergo an interesting and unusual targeting and translocation process was rather slow in coming, so that for many years not much effort was devoted to them. In addition, because of the small number of residues that are translocated across the bilayer, a major difficulty has been in establishing appropriate assays to investigate TA protein insertion. In our minds, the in vitro binding assays that have generally been used may not be adequate and may yield results that do not report on the events occurring in vivo. Indeed, tight binding of TA proteins to lipid bilayers without translocation of the COOH terminus can occur in artificial systems, as has been demonstrated for cytochrome b(5) (Dailey and Strittmatter, 1981). Thus, requirements for in vitro binding may not be the same as those for translocation of the COOH terminus. For this reason, we feel that TA protein insertion should be investigated using rigorous assays for COOH-terminal translocation. With this in mind, it should be possible to devise genetic screens in yeast as well as to conduct meaningful insertion studies in reconstituted systems in vitro. It is hoped that a combination of genetic and biochemical approaches will rapidly lead to the identification of the molecular components required for TA protein insertion, as occurred for other translocation systems some years ago.

Regulation of TA protein localization

Because of its unusual features, TA protein insertion is a potential target for novel regulatory mechanisms, and recent research has revealed that regulation can indeed occur. Here, we discuss two examples, both involving members of the Bcl-2 family.

TA proteins can reside constitutively in the cytosol and become integrated into a membrane in response to a signal. Although recruitment of peripheral proteins to the plasma membrane is a well-established mechanism in signaling pathways, the regulated integration of a transmembrane protein into the bilayer is probably unique to TA proteins. This phenomenon has been demonstrated for members of the Bcl-2 family, but may turn out to be of general significance.

Proteins of the Bcl-2 family are key regulators of apoptosis, promoting either cell survival (e.g., Bcl-2 and Bcl-X_L) or programmed cell death (e.g., Bax, Bad, Bak). The exact mechanisms by which these proteins work are debated (for review see Cory and Adams, 2002), but it is widely believed that oligomers of proapoptotic members contribute to the MOM permeabilization that allows efflux of apoptogenic proteins from the intermembrane space. The prosurvival members would have the opposite effect, antagonizing the proapoptotic members and preserving the MOM permeability barrier.

Most members of the Bcl-2 family are TA proteins, of which some reside in the cytosol and translocate to the MOM in response to an apoptogenic signal. Of these, proapoptotic Bax is the best studied example. In healthy cells, Bax resides in the cytosol in soluble, monomeric form. Early during apoptosis, Bax translocates from the cytosol to mitochondria where it disrupts the MOM permeability barrier (for review see Cory and Adams, 2002). Determination of the structure of Bax in solution has revealed that its COOH-terminal tail occupies a hydrophobic pocket thought to provide a binding site for the so-called BH3 domain of Bcl-2 family members that switch on the proapoptotic activity of Bax (Suzuki et al., 2000). Thus, displacement of the COOH-terminal region from the BH3 binding pocket, in addition to allowing the tail to interact with the MOM, could also promote dimer formation.

The sequence requirements for the regulated integration of Bax into the MOM have been investigated (Nechushtan et al., 1999). Essential for the regulated binding of Bax to the MOM is a Ser residue toward the end of the putative TMD (Ser184). When this residue is either deleted or replaced with Val or Ala, Bax constitutively localizes to the MOM. Moreover, the mutated tail region of Bax is sufficient to target a reporter (GFP) to the MOM. In contrast, GFP carrying the wild-type Bax tail remains cytosolic and is incapable of relocating to the MOM in response to an apoptogenic signal. Structural studies (Suzuki et al., 2000) revealed that an H-bond between Ser184 and Asp98 stabilizes the binding of the COOH-terminal tail in the hydrophobic pocket, explaining why deletion or substitution of this residue leads to dissociation of the tail, and subsequent integration into the MOM. However, it remains unclear why the wild-type tail, when appended to a reporter protein, does not associate with the MOM. Moreover, the nature of the stimulus causing the conformational switch that leads BAX to insert into the MOM is presently not understood. Given the wide interest in proteins of the Bcl-2 family, it is hoped that the exact events that trigger Bax's conformational switch will soon be discovered.

The distribution of TA proteins between the ER and the MOM might be regulated. As detailed earlier, upon release from ribosomes, TA proteins are targeted to a small subset of intracellular membranes, comprising the ER, the MOM, and, in plants, the plastid membrane. In a study aimed at elucidating the relation between ER and MOM targeting, we used a MOM-localized form of cytochrome b(5) modified to carry an N-glycosylation site close to the COOH terminus, as reporter for transit through the ER (Borgese et al., 2001). In vitro, this construct was capable of translocating its COOH terminus across ER microsomes, as demonstrated by the efficient utilization of the N-glycosylation consensus site. In vivo, however, it was targeted nearly exclusively to mitochondria and was not glycosylated, indicating that it did not transit through the ER. Thus, notwithstanding its full competence to insert into ER membranes, when faced with a choice in vivo, this protein preferred the MOM, suggesting that ER and MOM are in competition for TA proteins. This phenomenon could explain the dual MOM/ER localization of some TA proteins (Fig. 2). In addition, the outcome of the competition could well be modulated, for instance, by alteration of the chaperone repertoire within the cell or by posttranslational modifications of the TA protein itself. Such a regulated change of the target membrane could have important functional consequences.

An example of how localization and function of TA proteins could be modulated is offered by the antiapoptotic pro-

tein Bcl-2. Bcl-2 is targeted both to the ER and to the MOM, and association with membranes is required for its biological activity (for review see Cory and Adams, 2002). Studies with chimaeras have demonstrated that its membrane localization is mediated by the tail region (Nguyen et al., 1993), as is generally the case with TA proteins. In addition, interactions of the NH_2-terminal domain with a MOM-localized binding partner (FKB38) increase its mitochondrial localization (Shirane and Nakayma, 2003). Studies with forms of Bcl-2 engineered to localize exclusively to the ER or to the MOM have shown that in the two localizations it has different functions (Cory and Adams, 2002). More specifically, the ER associated form has been implicated in the maintenance of calcium homeostasis (He et al., 1997) and, although protective against a limited subset of apoptogenic signals, may in some cases be more effective than the MOM targeted protein. Thus, modulation of the targeting of Bcl-2, for instance by varying the expression levels of FKB38 (Shirane and Nakayma, 2003), could finetune the cell's response to death and survival signals.

Conclusions and perspectives

10 years ago a short review (Kutay et al., 1993) raised a number of questions on TA protein targeting and trafficking, for some of which we have clear answers today. As summarized in this review, we now know that the COOH terminus can be translocated across the bilayer, that these proteins are specifically targeted to a small subset of intracellular membranes, that the hydrophobic anchor plays a key role in targeting and trafficking to different organelles, and that insertion into the ER occurs via a novel mechanism probably not involving the classical translocation machinery. In addition, it has become apparent that TA protein targeting and insertion can be regulated, a finding that increases the interest in the mechanisms of localization of these proteins, many of which are key players in fundamental processes within cells. Nonetheless, the central issue of the nature of the molecules involved in TA protein insertion has not been resolved yet. Given the recent increased interest in TA proteins, it is hoped that answers to this question will be coming soon, opening the way to unravel novel modes of regulated trafficking of this class of membrane proteins.

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