Targeting mechanisms of tail anchored proteins in Yeast and mammalian cells

Bachelor thesis
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Date: 01-05-2017
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Abstract - Tail anchored (TA) proteins constitute a diverse group of proteins that share a distinct way of anchoring to biological membranes. Due to their topology TA proteins are obliged to use a post-translational mode of insertion. Since the discovery of TA proteins several research groups have tried to characterize the different targeting mechanisms that these proteins use. Until now several factors have been identified ranging from physicochemical properties of the transmembrane domain (TMD), to cytosolic chaperones that guide TA proteins to their correct membranes. Since TA proteins are located to several different locations within the cell, there is not a single pathway that is used by all TA proteins. Different locations use different mechanisms. This paper will give an overview of the targeting mechanisms, that have been identified so far, to the endoplasmic reticulum (ER), the mitochondrial outer membrane (MOM) and peroxisomes.

I. Introduction

Tail anchored (TA) proteins are a diverse group of membrane proteins, in eukaryotes, that share a distinct way of anchoring to biological membranes. TA proteins are characterized by a single transmembrane domain (TMD), close to the C-terminus, and a N-terminal functional domain. They are involved in a variety of cellular processes and can be found in essentially all membranes that are in contact with the cytosol. There are several membrane bound compartments facing the cytosol in eukaryotic cells and/so it is important that organellar proteins, such as TA proteins, are transported to the right membranes. Mislocalization of proteins can be detrimental for cell organelles. So protein targeting seems to be an essential feature of eukaryotic cells to ensure proper cellular organization. (Borgese et al., 2003; Opalinski et al., 2014)

So how do proteins reach their destination within the cell? The signal hypothesis provided an answer to this question. It was first hypothesized and demonstrated, by Blobel and Sabatini, for secretory and membrane proteins targeted to the ER. The signal hypothesis is based on the concept that information about the location and translocation is contained within the sequence of a protein. This signal sequence can be a permanent part of the protein, or a short-lived sequence that is removed when the protein has reached its destination. Besides the signal sequence there are several cytosolic and membrane bound factors that cause proteins to reach a specific destination within the cell. There are membrane bound receptors that are located on distinct cellular membranes and different cytosolic factors that are required to guide the proteins to their target membranes. One earliest and most well defined pathway is the sec61 pathway. The sec61 pathway is responsible for directing proteins to the ER; they can either remain in the ER or use the ER as an intermediate to reach other destinations within the cell. In this pathway the signal recognition particle (SRP) recognizes a hydrophobic signal sequence that is available at the ribosome precursor complex. When SRP binds to the hydrophobic signal sequence it slows down translation of the protein and interacts with the SRP receptor on the ER membrane. After the interaction with the SRP receptor the ribosome binds to the Sec61

translocon, which promotes elongation and integration of the protein into the ER. (Grudnik et al., 2009)

After the signal hypothesis was demonstrated to be true for ER targeted proteins, the signal hypothesis was shown to be true for proteins that were directed to other locations within the cell. The concept of signal-mediated targeting became the general theory that explains how proteins are brought to their correct location in (Matline, 2011)

There are different modes of translocation: co-translational and post-translational. Proteins that use the sec61 pathway are translocated co-translationally. They are synthesised as precursors on cytosolic ribosomes. Protein machineries recognize the precursor-ribosome complex and promote their translocation into the target organelle. However, this mechanism only works if the signal sequence is available before the protein is released from the ribosome. (Blobel et al., 1978; Borgese et al., 2003)

Thus, TA proteins cannot insert co-translationally since their signal sequence is located near the C-terminus of the protein (Fig. 1). Therefore TA proteins can only be translocated after translation is terminated. The targeting mechanisms for co-translational translocation are well defined, but this is not the case for TA protein targeting. TA proteins have recently become an interesting research topic due to their variety of functions and locations within the cell (Table 1). An important question in this research is how TA proteins are transported from the cytosol to their target membranes. (Borgese et al., 2007; Buentzel et al., 2015)

Although TA proteins can be found in virtually all membranes in the cell, they cannot insert directly into all membranes. They can only insert into a limited number of membranes, other destinations have to be reached indirectly by first inserting into the ER and membrane trafficking. Targets for direct insertion of TA proteins are: the mitochondrial outer membrane (MOM), the chloroplast envelope, the endoplasmic reticulum (ER) and peroxisomes. (Borgese, 2011) TA proteins cannot be inserted directly into membranes of other organelles in the secretory pathway. This is attributed to the requirement of a certain lipid composition for direct insertion of TA proteins. (Beilharz et al., 2003)

Due to their distinct topology TA proteins cannot use conventional targeting mechanisms to reach their destination in the cell. Since the discovery of TA proteins their targeting mechanisms have been a topic of interest. In recent years a lot of progress has been made in finding TA protein targeting mechanisms. Some important findings are organelle specific signal sequences and the presence of cytosolic chaperones to mediate TA protein insertion. This paper will give an overview of the available knowledge about TA protein targeting mechanisms. So far only a couple of organelles have been identified that are capable of direct insertion of TA proteins. Namely: the MOM, ER, chloroplast outer envelope and peroxisomes. The scope of this paper is limited to yeast and mammalian TA proteins. So only the mechanisms that are used to direct TA proteins to the MOM, ER and peroxisomes will be discussed.

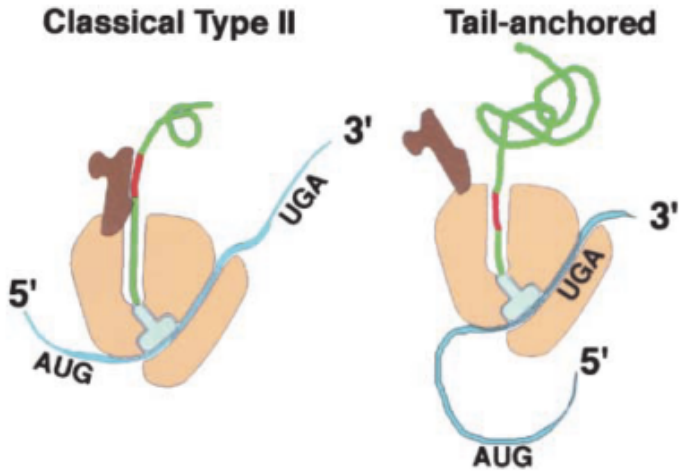

Figure 1. **Membrane insertion of TA proteins happens post-translationally.** This figure shows why TA-proteins cannot insert into membranes co-translationally. In contrast to type two proteins, the signal sequence will only become available after the protein is released from the ribosome. In classical type two proteins (defined as proteins with a single TMD with a cytosol facing N-terminal and exoplasmic C-terminal which are delivered to the ER in a SRP dependent co-translational pathway) the signal sequence will be recognized by SRP during translation and will be guided to the target membrane for insertion. Source: Borgese et al., 2003

Target membrane	TA protein	Function	Notes
ER	Sec61β	Protein translocation	
	Ramp4	Protein translocation	
	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
	Sed5	Golgi SNARE (Yeast orthologue of mammalian syntaxin 5)	Transported from the ER to the Golgi complex
	Cyt b5	Lipid metabolism in the ER	
OMM	Small TOM proteins	Protein translocation	
	OMb5	Enzymatic	Mitochondrial isoform of cyt b5
	VAMP-1B	SNARE of unknown function	Splicing variant of ER-targeted isoform (VAMP, or synaptobrevin-1A)
	Bcl-XL, Bak	Regulation of apoptosis	
COE	*A. thaliana* cyt b5 isoform At1g26340	Enzymatic	
	Outer Envelope Membrane Protein 9	Unknown	
	Toc 33, 34	Protein translocation	
Peroxisomes	Pex26	Peroxisome biogenesis	
Dual targeting: ER and OMM	Bcl-2	Regulation of apoptosis	
Dual targeting: OMM and peroxisomes	Fis1	Mitochondrial and peroxisomal fission	

Table 1. **Different functions and localizations of TA-proteins.** Source: Borgese et al., 2011
Abbreviations: OMM Outer mitochondrial membrane, COE chloroplast outer envelope, ER Endoplasmic reticulum

II. Targeting of TA proteins to the ER

After the discovery of TA proteins and their distinct way of translocation, the question arose whether they use the same targeting mechanisms as other membrane proteins. As mentioned earlier, TA proteins can insert directly into several target membranes. The information about which membrane they have to be inserted into is contained in the C- terminal region of the protein. This was discovered by deletion mutagenesis and by fusing fluorescent tags to N-terminus of TA proteins.(Kim et al., 2009; Kim et al., 2015; Hawthorne et al., 2016)
The tail segment of TA proteins consists of a single TMD and a short luminal domain. The hydrophobicity of the TMD and the charge of the flanking residues play a crucial role in determining between the ER and the MOM. (Abell et al., 2007;Beilharz et al., 2003; Borgese et al., 2003)

The ER is an important target for TA proteins. There are TA proteins that remain in the ER, but there the ER is also the starting point for many TA proteins that are destined for other organelles in the cell. They are first inserted into the ER before they are transported via the secretory pathway to their final destination. So many TA proteins contain a signal sequence that will direct them to the ER.

The tail segment of ER directed TA proteins generally contain a TMD with moderate hydrophobicity. The hydrophobicity of the TMD is also important for the mode of insertion. Some ER directed TA proteins could insert into the ER unassisted. For example Cytochrome b5 (Cytb5) can insert without needing any cytosoloc or membrane bound factors. This was shown *in vitro* where cytb5 could translocate into pure lipid vesicles without any assistance. (Kim et al., 1997) It was shown that this unassisted translocation was due to the low hydrophobicity of the TMD. When the hydrophobicity cytb5's TMD was increased it lost the ability of unassisted insertion. Mixing the residue order of the TMD did not affect this, so the TMD hydrophobicity is solely responsible. This unassisted pathway is not available to all ER targeted TA proteins. (Brambillisca et al., 2006) Brambillasca et al. hypothesized that molecular chaperones prevent TA proteins with more hydrophobic TMD's from aggregating. They suspect that the mechanism of insertion remains the same. (Brambillasca et al., 2006)

Besides preventing aggregation chaperones can also create more specificity through unique interactions with distinct membrane bound receptors. Although the hydrophobicity of the TMD provides us with some information about where a TA protein goes, it is not sufficient to determine the exact location. In general ER

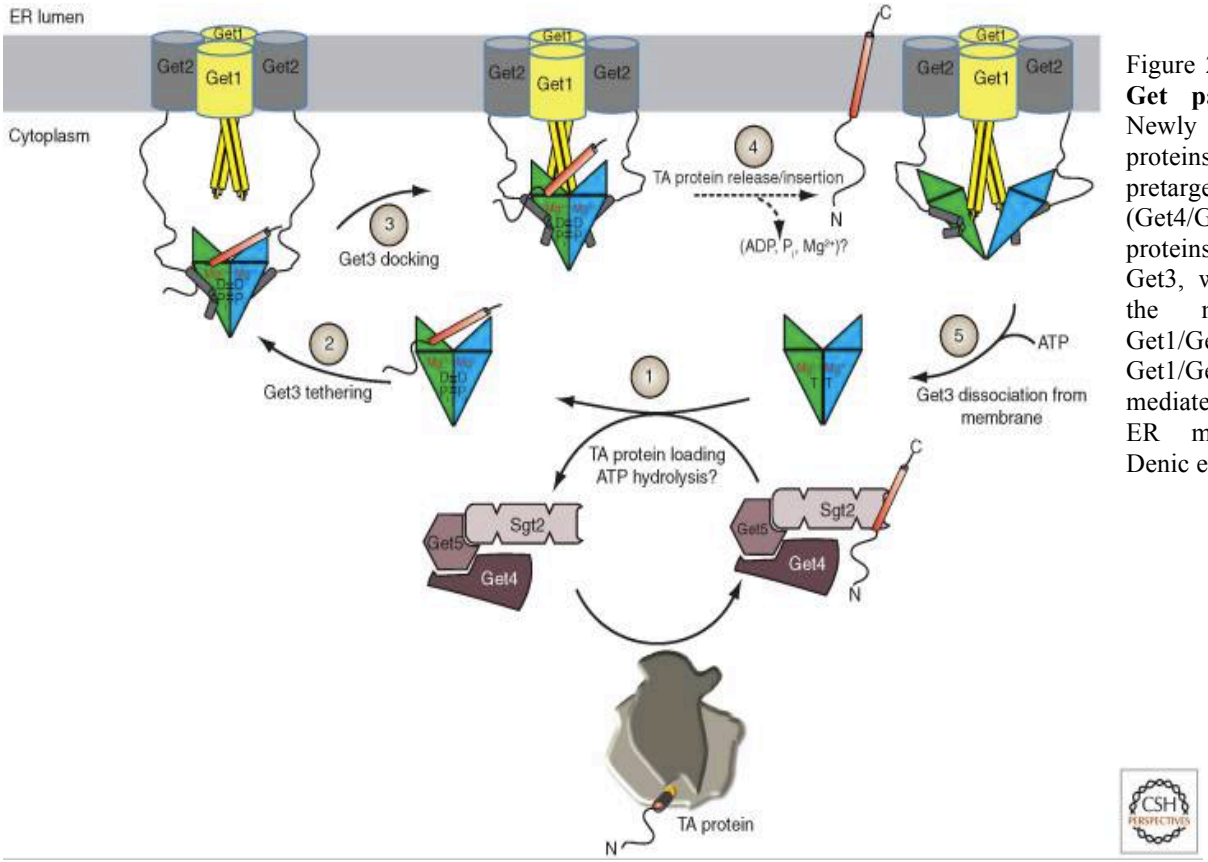

Figure 2. **Overview of the Get pathway in yeast**. Newly synthesized TA proteins are captured by the pretargeting complex (Get4/Get5/Sgt2). The TA proteins are transferred to Get3, which shuttles it to the membrane bound Get1/Get2 complex. The Get1/Get2 complex mediates insertion into the ER membrane. Source: Denic et al., 2013

proteins got a moderately hydrophobic TMD, but the range of hydrophobic TMD's that the ER accepts is quite large. It also overlaps with TA proteins for other organelles. There are TA proteins that are targeted to the MOM that have comparable TMD hydrophobicity to ER targeted proteins. (Beilharz et al., 2003; Abell et al., 2007) So other factors, like chaperones, are necessary to account for further targeting specificity.

Chaperones recognize a specific sequence in the protein. They can bind this sequence and than escort the protein to the target membrane. Once they are there the chaperone-protein complex can interact with a membrane bound receptor, which promotes release from the chaperone and integration into the membrane. (Abell et al., 2007)

As mentioned in the introduction, most ER proteins translocate into the ER post-tranlationally using the sec61 translocon. However, TA proteins can insert into the ER without the assistance of the sec61translocon. There are studies that suggest that SRP still has a function in trafficking TA proteins to their right membrane.(Abell et al., 2007)

But TA proteins can still translocate in the ER in absence of SRP. (Steel et al., 2002) When SRP delivers its protein to the ER it binds with the SRP receptor. In order to release the polypeptide and promote its integration into the ER GTP is needed. However experiments have shown that most TA proteins can target the ER in a GTP independent and ATP dependent manner. Up to know several chaperones have been identified that interact with ER targeted TA proteins. Abell et al. have shown that Hsc70 and Hsp40 are sufficient ATP dependent integration of sec61beta, a TA protein that resides in the ER. Another central player in the ATP dependent insertion of TA proteins into the ER is the GET complex. Removal of some of the key proteins of the Get complex leads to a range of defects associated with mislocalization of TA proteins. (Shuldiner et al., 2008)

Protein		Role in pathway
S. cerevisiae	Mammals	
Get3	TRC40	ATPase that binds the transmembrane domain and delivers TA substrates to the ER membrane.
Get1	Tryptophan Rich Basic Protein (WRB)	Transmembrane protein of the ER. In *Saccharomyces* the Get1/2 complex constitutes the Get3 Receptor; a similar role for the mammalian homologue has not been demonstrated.
Get2	–	Transmembrane protein of the ER. In *Saccharomyces* the Get1/2 complex constitutes the Get3 Receptor.
Get4	Conserved Edge expressed protein (cee)/TRC35	Get4/5 complex is thought to deliver TA proteins from the ribosome to Get3. In mammals, it is associated with a third component, Bat3.
Get5/Mdy2	Ubl4A	Ubl domain containing protein. Get4/5 complex is thought to deliver TA proteins from the ribosome to Get3. In mammals, it is associated with a third component, Bat3.
–	Bat3	Ubiquitin-like and BAG domain containing protein. Acts upstream to TRC40.
Sgt2	SGTA	Tetratricopeptide repeat peptide-containing protein that interacts with Get4/5. Precise role in TA protein biogenesis not understood.

Table 2. **Proteins involved in the Get pathway, in yeast, and their counterparts in the TRC40 pathway, in mammalian cells.** Source: Borgese et al, 2011

Cross-linking experiments also showed that ER targeted TA proteins interact with Get3, a cytosolic protein in the Get pathway. The Get pathway starts with a newly synthesized TA protein. A pretargeting complex (consisting of Sgt2, Get4 and Get5) recognizes the TMD. Besides capturing the protein it also shields the TMD from the aqueous environment, which prevents aggregation. Get3 binds the TA protein and acts like a shuttle before interacting with the membrane bound Get1/Get2 complex.
(Fig2) (Abell et al., 2007; Denic et al., 2013; Schuldiner et al., 2008)

How the TA protein inserts into the membrane is still unclear, but during their interaction the TA protein is released from Get3 and inserts into the bilayer. That the Get complex mediates insertion of TA proteins into the ER has been discovered in yeasts. These Get proteins are not found in mammalian cells, but they have their counterparts that fulfil the same functions. TRC40 has been identified as a structural and functional homologue of Get3 in mammalian cells. (Stefanovic & Hegde, 2007)
The receptor Get1 also has a counterpart in mammalian cells. WRB has been shown contain sequence and functional similarities with Get1. Table2 gives an overview of the proteins involved in the Get pathway and their counterparts in mammalian cells. (Denic et al. 2013; Vilardi et al., 2011; Voth et al., 2014)

The ER can be the final destination for TA proteins, but there are also many TA proteins that have to be transported further down the secretory pathway or have to be localized on a specific place in the ER membrane. The moderate hydrophobicity of the TMD appears to be enough to ensure insertion into the ER, but what determines where they go after that. Beilharz et al. showed that further localization within the ER mebrane or to other organelles happens through a second signal sequence located in the cytosolic segment of ER TA proteins. There is a two-step mechanism to ensure correct targeting for ER TA proteins. The first step is the TMD, which promotes insertion into the ER. The second step determines where the protein goes once it is inserted into the ER.
PRM3, a TA protein that is localized in the nuclear envelope, is first inserted into the ER. After insertion into the ER a short sequence on the cytosolic domain, the nuclear localization sequence NLS, is recognized by RAN, which promotes localization in the nuclear envelope. To see if this was the only requirement for localization in the nuclear envelop, they removed the TMD and made PRM3 a cytosolic protein. This cytosolic protein was also localized in the nuclear envelope. (Beilharz et al., 2003)

III. Targeting of TA proteins to the MOM

Another important destination for TA proteins, which cannot be reached through the ER, is the MOM. In contrast to ER proteins, most mitochondrial proteins are translocated post-translationally. Most proteins that are targeted to the MOM do not have a cleavable N- terminal

signal sequence, but an internal uncleavable signal sequence. The TOM machinery (translocase of the outer membrane) is thought to be responsible for integrating virtually all MOM targeted proteins. (Ahting et al., 2005) However, MOM targeted TA proteins can insert into the MOM without the assistance of the TOM machinery. In cells depleted of components of the TOM machinery TA proteins were shown to still insert correctly into the MOM. (Setoguchi et al., 2006) Just like ER targeted TA proteins, MOM targeted TA proteins share a signal sequence in their tail segment. Besides a moderately hydrophobic TMD, MOM TA proteins also have TMD flanking residues that are rich in basic amino acids. By fusing GFP with the tail segment of MOM targeted TA proteins it was discovered that this was sufficient to ensure targeting of the MOM. (Nechustan et al., 1999; Setoguchi et al., 2006) Even in absence of any cytosolic factors these fused GFP-TA proteins were still able to insert correctly into the MOM. However some MOM TA proteins still depent on other cytosolic factors to integrate into the ER. But these factors are not common for all MOM TA proteins and are protein specific. When MOM TA proteins are over expressed they compete with each other to be integrated into the MOM, indicating that they share a common pathway. (Horie et al., 2002; Setoguchi et al., 2006) Since they share a common pathway the function of chaperones are attributed to proper folding and making the TMD available of specific proteins. It is still uncertain if MOM TA proteins share a membrane bound component that recognizes MOM TMD's and mediate integration into the membrane. However the TOM machinery is not responsible for the integration of MOM TA proteins. (Egan et al., 1999; Setoguchi et al., 2006)

IV. Targeting of TA proteins to peroxisomes

Besides the MOM and the ER, peroxisomes are also an important target for TA proteins. The tail segments of peroxisomal TA proteins have comparable physicochemical properties as MOM targeted TA proteins. This could explain why some peroxisomal TA proteins can be found in the MOM when they are mislocalized. There are mechanisms in place that remove mislocalized peroxisomal TA proteins from the MOM. However, it is very unlikely that MOM and peroxisomal TA proteins do not discriminate between the MOM and the ER and their localization only depends on removal from a wrongly targeted organelle. And indeed this does not appear to be the case. There are distinct targeting pathways for peroxisomal TA proteins that ensure their correct targeting to peroxisomes. In the next chapter there will be an overview of the current knowledge about targeting mechanisms for peroxisomal TA proteins. As model proteins Pex15 and Pex26 will be used. These proteins are both responsible for the recruitment of AAA peroxins to the peroxisomal membrane, but Pex15 is present in yeasts and Pex26 in mammals. Despite their similar function they are not homologs and both use a different targeting mechanism to reach peroxisomes. (Borgese et al., 2011; Halbach et al., 2006; Zand et al., 2010)

Peroxisomal TA proteins can reach their destination through a variety of different routes. Some of these routes are indirect and go through the ER, but there is also evidence that peroxisomal TA proteins can be targeted directly from the cytosol. All these routes rely on chaperones and other import machineries, unassisted insertion of peroxisomal TA proteins has not been reported yet. So far two import routes for peroxisomal TA proteins have been identified. Pex15 was shown to initially target the ER using the Get pathway. After insertion into the ER it reaches the peroxisomes through membrane traffic. Get3 recognizes the tail segment of Pex15 and guides it to the ER. (Shuldiner et al., 2008)

This is also supported by the fact that Pex15 is mislocalized in mitochondria in yeast strains that lack a functional Get pathway. Although Pex26 has a similar function as Pex15, uses a more direct route to reach peroxisomes than Pex15. After translation Pex26 is recognized and bound by Pex19 in the cytosol. Pex19 can than be recognized by Pex3, a peroxisomal membrane protein, which mediates insertion into the peroxisomal membrane. (Abell et al., 2007; Buentzel et al., 2015) The targeting information

for both Pex15 and Pex26 is contained in their C terminal region. The Get3 binding site alone in Pex15 cannot be held accountable for the targeting to peroxisomes, since this binding site is also present in other TA proteins going through the ER. Besides the binding site for Get3 there are also two Pex19 binding sites in Pex15. One overlapping the TMD and one in the luminal segment of the tail. These binding sites are also present in Pex26. When Pex26 is expressed in yeast it follows the Get pathway just like Pex15. This shows that the signal sequence is conserved between mammals and yeasts and that the mode of transport depends on the chaperones available in a certain organism. (Buentzel et al., 2015; Chen et al., 2014)

Pex19 has a chaperone function in the direct insertion of Pex26 into peroxisomes. However, correct targeting of Pex15 is also dependent on Pex19. In Pex19 depleted yeast strains Pex15 remains in the ER. So Pex19 has a role in targeting Pex15 after it went through the Get pathway. This is in line with the idea that TA proteins that go through the ER rely on a two stage targeting signal. The first signal directs them to the ER and the second causes localization to the target membrane. The function of Pex19 in this process is not that of a protein chaperone anymore, since Pex15 is already integrated into a membrane. Instead Pex19 is implicated as a sorting receptor for the formation of pre-peroxisomal vesicles on the ER. Pex19 and Pex3 are both required for budding pre-peroxisomal vesicles from the ER in yeast. It was recently discovered that Pex19 and Pex3 also play an important role in budding of peroxisomal protein containing vesicles from the ER. (Agrawel et al., 2016; Lam et al., 2010)

This was shown for ring-domain and docking complex proteins destined for peroxisomes. This has not been shown for Pex15 yet, but it does confirm the hypothesis that Pex19 could be important for vesicular transport of Pex15 from the ER. (Agrawel et al., 2016; Chen et al. 2014)

As mentioned earlier Pex15 and Pex26 both contain two binding domains for Pex19. It is already established that Pex19 works as a chaperone that guides Pex26 to Pex3 on peroxisomes. One of the binding sites overlaps with the TMD and one is contained in the luminal domain. For Pex26 this luminal domain appears to be sufficient for targeting to peroxisomes. Pex15 shows a conserved targeting sequence and also contains a Pex19 binding site in the luminal domain. When this binding site is fused to Fis1 (a mitochondrial TA protein) it will redirect Fis1 to peroxisomes. The TMD alone is thought to be the ER targeting signal for Pex15. And the Pex19 binding site on the luminal domain contains targeting information for peroxisomes. (Chen et al., 2014; Yagita et al., 2013) These findings confirm that the signal sequence of Pex15 and Pex26 is conserved and explains why Pex15 uses the same route as Pex26 when it is expressed in mammalian cells. Nonetheless it seems unlikely that these binding sides also function in normal Pex15 transport through the Get pathway. The two binding sites of Pex15 overlap with the TMD and the luminal domain. So once Pex15 is inserted into the ER they should not be available anymore for cytosolic chaperones. To see if Pex19 binds to Pex15 before it enters the ER and opsin tag was attached to the luminal domain of Pex15. Once the luminal domain is correctly integrated into the ER this tag will get glycosylated. In both wt and Pex19 deletion strains there was no significant difference in the amount of glycosylated Pex15. This indicates that Pex19 has no significant function before Pex15 is inserted into the ER. (Borgese et al., 2011; Yagita et al., 2013)

The direct and indirect pathways above describe the major targeting routes for TA proteins in mammalian cells and yeast respectively. It appears that there are several mechanisms in place for the transport of peroxisomal TA proteins and that they are not mutually exclusive. When one route is compromised the other one can take over. For example when Get3 is removed a small portion of Pex15 can still be targeted to peroxisomes through a Pex19/Pex3 dependent pathway. The possibility of multiple targeting routes for peroxisomal TA proteins makes it difficult to determine which route is responsible. This could also explain the conflicting views on how peroxisomal TA proteins are transported.

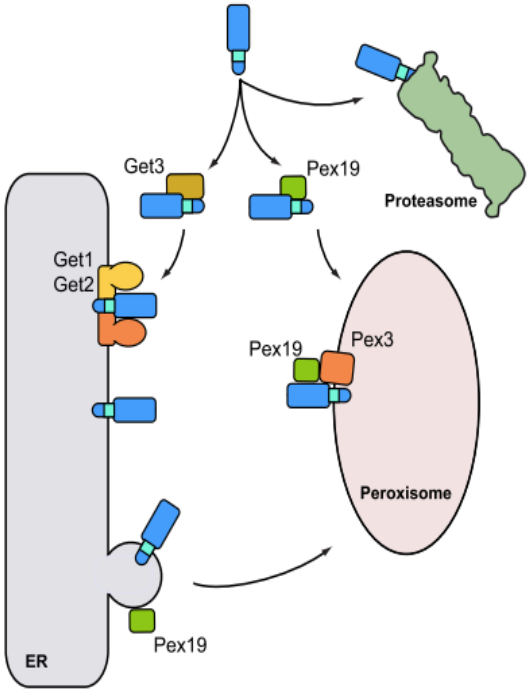

Figure 3. **Model of peroxisomal TA protein targeting**. This figure shows the different pathways peroxisomal TA proteins can use to reach the peroxisome. Peroxisomal TA proteins will be inserted directly into the ribosome when they bind Pex19 directly after release form the ribosome. When they bind Get3/TRC40 they will follow an indirect route through the ER. Pex19 has an essential role in both pathways. In the direct pathway it functions as a cytosolic chaperone. In the indirect pathway Pex19 is required to ensure budding from the ER. Source: Buentzel et al., 2015

V. Conclusion and Discussion

In recent years a lot of progress has been booked in uncovering targeting mechanisms for TA proteins. *In vitro* studies have shown that some TA proteins can insert unassisted into bilayers. (Kim et al., 1997) So to prevent opportunistic insertion into the wrong membrane, there have to be organelle specific targeting mechanisms that ensure insertion into the right membrane. So far several distinct targeting pathways have been identified. It was soon discovered that the tail segment played an essential role as a signal sequence. The hydrophobicity of the TMD and the charge of the flanking residues have been identified as critical determinants in TA protein targeting. The tail segment can account for some specificity, but the differences in the tail segment between differently located TA proteins can sometimes be quite minor. Another important contribution to targeting specificity is achieved by cytosolic chaperones.

For ER TA proteins several chaperones have been identified such as Hsc70, Hsp40, Get3 and SRP in some cases. Get3 is the cytosolic chaperone in the Get pathway. The Get pathway is one of the major routes for TA proteins in yeast. Mammalian cells also have a similar pathway with TRC40 as molecular chaperone. Some TA proteins remain in the ER, but the ER is also a gateway for other organs. TA proteins can reach other locations in the cell through membrane traffic. Beilharz et al**.** showed that there could be a two- stage mechanism and that there should be a second signal sequence, possibly on the cytosolic domain, to determine where it has to go further down the secretory pathway. For MOM targeted proteins it is still unclear if there is a shared pathway, like the Get pathway in the ER. There are MOM TA proteins that depend on other factors for their insertion into the MOM, but these highly protein specific and are thought to be necessary for presenting the TMD to the bilayer. Until now there are no chaperones detected that are shared by all MOM TA proteins. And targeting of the MOM only depends on the tail segment of TA proteins and the absence of other chaperones interacting with them.

Another interesting discovery is that there is a conserved signal sequence between Pex15 and Pex26. These functional homologs both use different routes to reach peroxisomes in yeast and mammalian cells respectively. When Pex26 is expressed in yeast it follows the Get pathway just like Pex15. And when Pex15 is expressed in mammalian cells it favours direct insertion, mediated by Pex19 and Pex3. Both contain a similar C terminal segment with two Pex19 binding sites, but the rest of the protein shows very little sequence similarities. In Pex26 the luminal binding site is essential for proper localization. I consider it unlikely that Pex19 also binds to Pex15 when it is already inserted into the ER, since both binding sites will be hidden in the membrane or the ER lumen. It still remains a question whether these conserved binding sites

are a product of divergent evolution, where Pex15 did not lose the Pex19 binding sites, or convergent evolution where Pex15 binding sites for a "back-up" direct TA targeting pathway. (Beuntzel et al., 2015)

I think that these findings demonstrate that TA proteins targeting is a complex process where multiple factors determine where a proteins goes. A combination of biochemical factors, such as TMD hydrophobicity and lipid composition of certain membranes, and the presence of chaperones can account for the specificity of TA protein targeting. The most appealing view for me is that after synthesis there is a constant competition between chaperones. The chaperone that binds the fastest and most stable determines where the TA protein goes. I think this is in line with the findings about Pex15 and Pex26. When they are expressed in different organisms they can take another targeting route when their native chaperone is not present.

So far TA protein targeting mechanisms have been an important topic for several research groups over the past couple of decades. A lot has been clarified about how they reach their target organelles within the cell. However, there are still questions that remain to be answered about their targeting mechanisms. For further research it would be interesting to find out how TA proteins are transported to their final destination after they are inserted into the ER. Beilharz et al. already identified bipartite signals for some TA proteins. For peroxisomal TA proteins it is still unclear how they are sorted from the ER to the peroxisomes. There seems to be a dependency on Pex19, but how this exactly works is still unclear. This would also be an interesting research topic for TA proteins that are inserted into the ER to reach other membranes in the secretory pathway.

References

Abell, B. M., Rabu, C., Leznicki, P., Young, J. C., & High, S. (2007). Post-translational integration of tail-anchored proteins is facilitated by defined molecular chaperones. *Journal of Cell Science*, *2*. http://doi.org/10.1242/jcs.002410

Agrawal, G., Fassas, S. N., Xia, Z. J., & Subramani, S. (2016). Distinct requirements for intra-ER sorting and budding of peroxisomal membrane proteins from the ER. *Journal of Cell Biology*, *212*(3), 335–348. http://doi.org/10.1083/jcb.201506141

Ahting, U., Waizenegger, T., Neupert, W., & Rapaport, D. (2005). Signal-anchored Proteins Follow a Unique Insertion Pathway into the Outer Membrane of Mitochondria *. *JBC*, *280*(1), 48–53. http://doi.org/10.1074/jbc.M410905200

Beilharz, T., Egan, B., Silver, P. A., Hofmann, K., & Lithgow, T. (2003). Bipartite Signals Mediate Subcellular Targeting of Tail-anchored Membrane Proteins in Saccharomyces cerevisiae *. *The Journal of Biological Chemistry*, *278*(10), 8219–8223. http://doi.org/10.1074/jbc.M212725200

Borgese, N., Brambillasca, S., & Colombo, S. (2007). How tails guide tail-anchored proteins to their destinations. *Current Opinion in Cell Biology*, *19*(4), 368–375. http://doi.org/http://dx.doi.org/10.1016/j.ceb.2007.04.019

Borgese, N., Colombo, S., & Pedrazzini, E. (2003). The tale of tail-anchored proteins : coming from the cytosol and looking for a membrane. *The Journal of Cell Biology*, *161*(6), 1013–1019. http://doi.org/10.1083/jcb.200303069

Borgese, N., & Fasana, E. (2011). Targeting pathways of C-tail-anchored proteins. *BBA - Biomembranes*, *1808*(3), 937–946. http://doi.org/10.1016/j.bbamem.2010.07.010

Brambillasca, S., Yabal, M., Makarow, M., & Borgese, N. (2006). Unassisted translocation of large domains across phospholipid bilayers. *The Journal of Cell Biology*, *175*(5), 767–777.

Buentzel, J., Vilardi, F., Lotz-havla, A., Gärtner, J., & Thoms, S. (2015). Conserved targeting information in mammalian and fungal peroxisomal tail-anchored proteins. *Scientific Reports*, *5*, 1–14. http://doi.org/10.1038/srep17420

Chen, Y., Pieuchot, L., Loh, R. A., Yang, J., Mahfuzh, T., Kari, A., … Jedd, G. (2014). Hydrophobic handoff for direct delivery of peroxisome tail-anchored proteins. *Nature Communications*. http://doi.org/10.1038/ncomms6790

Denic, V., Do, V., & Sinning, I. (2013). Endoplasmic Reticulum Targeting and Insertion of Tail-Anchored Membrane Proteins by the GET Pathway. *Cold Spring Harbor Perspectives in Biology*, 1–11.

Egan, B., Beilharz, T., George, R., Isenmann, S., Gratzer, S., Wattenberg, B., & Y, T. L. (1999). Targeting of tail-anchored proteins to yeast mitochondria in vivo. *FEBS Lett.*, *451*(3), 243–248.

Grudnik, P., Bange, G. & Sinning, I. (2009). Protein targeting by the signal recognition particle. *Biological Chemistry*, 390(8), pp. 775-782. Retrieved 1 May. 2017, from doi:10.1515/BC.2009.102

Halbach, A., Landgraf, C., Lorenzen, S., Rosenkranz, K., Volkmer-engert, R., Erdmann, R., & Rottensteiner, H. (2006). Targeting of the tail-anchored peroxisomal membrane proteins PEX26 and PEX15 occurs through

C-terminal PEX19-binding sites. *Journal of Cell Science*, *119*, 2508–2517. http://doi.org/10.1242/jcs.02979

Hawthorne, J. L., Mehta, P. R., Singh, P. P., Wong, N. Q., & Quintero, O. A. (2016). Positively Charged ResiduesWithin the MYO19 MyMOMA Domain Are Essential for Proper Localization of MYO19 to the Mitochondrial Outer Membrane. *Cytoskeleton*, *73*, 286–299. http://doi.org/10.1002/cm.21305

Horie, C., Suzuki, H., Sakaguchi, M., & Mihara, K. (2002). Characterization of Signal That Directs C-Tail – anchored Proteins to Mammalian Mitochondrial Outer Membrane. *Molecular Biology of the Cell*, *13*(May), 1615–1625. http://doi.org/10.1091/mbc.01

Jones, J. M., Morrell, J. C., & Gould, S. J. (2004). PEX19 is a predominantly cytosolic chaperone and import receptor for class 1 peroxisomal membrane proteins. *The Journal of Cell Biology*, *164*(1), 57–67. http://doi.org/10.1083/jcb.200304111

Kim, P. K., & Hettema, E. H. (2015). Multiple Pathways for Protein Transport to Peroxisomes. *Journal of Molecular Biology*, *427*(6), 1176–1190. http://doi.org/10.1016/j.jmb.2015.02.005

Kim, P. K., Janiak-spens, F., Trimble, W. S., Leber, B., & Andrews, D. W. (1997). Evidence for Multiple Mechanisms for Membrane Binding and Integration via Carboxyl-Terminal Insertion Sequences †. *Biochemistry*, *2960*(97), 8873–8882.

Kim, P. K., Trimble, W. S., Leber, B., & Andrews, D. W. (1999). Identification of the Endoplasmic Reticulum Targeting Signal in Vesicle-associated Membrane Proteins *. *The Journal of Biological Chemistry*, *274*(52), 36876–36882.

Lam, S. K., Yoda, N., Shekman, R., & Sabatini, D. D. (2010). A vesicle carrier that mediates peroxisome protein traffic from the endoplasmic reticulum. *PNAS*, *107*(50), 21523–21528.

Matlin, K. S. (2011). Spatial expression of the genome: the signal hypothesis at forty. *Nat Rev Mol Cell Biol*, *12*(5), 333–340. Retrieved from http://dx.doi.org/10.1038/nrm3105

Nechushtan, A., Smith, C. L., Hsu, Y., & Youle, R. J. (1999). Conformation of the Bax C-terminus regulates subcellular location and cell death. *The EMBO Journal*, *18*(9), 2330–2341.

Opalinski, L., Becker, T., & Pfanner, N. (2014). Clearing tail-anchored proteins from mitochondria. *PNAS*, *111*(22), 1–2. http://doi.org/10.1073/pnas.1406864111

Schuldiner, M., Metz, J., Schmid, V., Denic, V., Rakwalska, M., Dieter, H., … Weissman, J. S. (2008). The GET Complex Mediates Insertion of Tail-Anchored Proteins into the ER Membrane. *Cell*, *134*(4), 634–645. http://doi.org/10.1016/j.cell.2008.06.025

Setoguchi, K., Otera, H., & Mihara, K. (2006). Cytosolic factor- and TOM-independent import of C-tail-anchored mitochondrial outer membrane proteins. *The EMBO Journal*, *25*(24), 5635–5647. http://doi.org/10.1038/sj.emboj.7601438

Steel, G. J., Brownsword, J., & Stirling, C. J. (2002). Tail-Anchored Protein Insertion into Yeast ER Requires a Novel Posttranslational Mechanism Which Is Independent of the SEC Machinery †. *Biochemistry*, *41*, 11914–11920.

Stefanovic, S., & Hegde, R. S. (2007). Identification of a Targeting Factor for Posttranslational Membrane Protein Insertion into the ER. *Cell*, *128*, 1147–1159. http://doi.org/10.1016/j.cell.2007.01.036

Vilardi, F., Lorenz, H., & Dobberstein, B. (2011). WRB is the receptor for TRC40 / Asna1-mediated insertion of tail-anchored proteins into the ER membrane. *Journal of Cell Science*, *124*(8), 1301–1307. http://doi.org/10.1242/jcs.084277

Voth, W., Schick, M., Gates, S., Li, S., Vilardi, F., Southworth, D. R., … Jakob, U. (2014). The protein targeting factor GET3 functions as an ATP-independet chaperone under oxidative stress conditions. *Mol Cell.*, *56*(1), 116–127. http://doi.org/10.1016/j.molcel.2014.08.017.

Yagita, Y., Hiromasa, T., & Fujiki, Y. (2013). Tail-anchored PEX26 targets peroxisomes. *The Journal of Cell Biology*, *200*(5), 651–666. http://doi.org/10.1083/jcb.201211077

Zand, A. Van Der, Braakman, I., & Tabak, H. F. (2010). Peroxisomal Membrane Proteins Insert into the Endoplasmic Reticulum. *Molecular Biology of the Cell*, *21*, 2057–2065. http://doi.org/10.1091/mbc.E10