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## The Special Delivery of a Tail-Anchored Protein: Why it Pays to Use a Dedicated Courier

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### Abstract

The membrane-spanning C-terminal regions in tail-anchored proteins must be recognized and delivered post-translationally to the endoplasmic reticulum or mitochondrial membrane. A paper in this issue (Wang et al., 2010) and another recent report (Mariappan et al., 2010) delineate early steps in this pathway.

Cell biology textbooks indicate that proteins targeted to the secretory pathway interact first with the endoplasmic reticulum (ER). This event requires recognition of an N-terminal signal sequence on an elongating polypeptide chain by the signal recognition particle, SRP (Fig. 1A). SRP binding slows the rate of translation, enabling efficient delivery of a ribosome-nascent polypeptide chain complex to the ER. At the ER membrane, SRP is released, translation resumes, and the polypeptide is threaded into the Sec61 translocation pore and into the ER lumen, if the protein is soluble, or into the ER membrane if the protein contains transmembrane domains. What most cell biology textbooks don't teach us—at least in detail—is that soluble proteins can also enter the secretory pathway post-translationally with the help of cytoplasmic chaperones (Fig. 1B). In yeast, the choice between the co-translational (SRP-dependent) and post-translational pathway is dictated by the hydrophobic core of the N-terminal signal sequence (Ng et al., 1996). Yet another class of post-translationally targeted proteins includes those with C-terminal hydrophobic motifs. This tail anchor (TA) transmembrane sequence somehow finds its way to the ER and then slips into the lipid bilayer in a Sec61-independent manner. Some of these TA proteins are then delivered to later compartments of the secretory pathway by vesicle transport. Other TA proteins are targeted directly to the mitochondrial membrane post-translationally. So, how does a given TA “know” into which membrane it must insert? An answer to this question is of profound importance given that ~3% of the eukaryotic genome encodes TA proteins, and TA proteins are required for a diverse array of cellular functions (Wattenberg and Lithgow, 2001; Borgese et al., 2007). In this issue of *Molecular Cell* (Wang et al., 2010), Denic and colleagues report on the identification of a protein complex in yeast that receives TA proteins soon after synthesis. Included within this complex is a long-sought factor that helps dictate the initial cellular residence of TA proteins.

Previous ground-breaking studies by the Weissman and Hegde laboratories identified a conserved, cytoplasmic ATP-hydrolyzing protein that recognizes TA sequences after their synthesis and release from the ribosome. The protein is known as Get3 (Guided Entry of TA

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proteins) in yeast, and the homolog is TRC40 (Transmembrane domain Recognition Complex) in mammals (Stefanovic et al., 2007; Schuldiner et al., 2008). Get3 transfers its cargo to the Get1-Get2 complex in the ER membrane (Fig. 1C). Indeed, a chaperone-like protein that acts at this step may be necessary to prevent the TA sequence from aggregating. However, it has remained unknown whether a protein complex acts upstream of Get3, which like SRP would similarly chaperone a newly synthesized protein during membrane targeting.

To this end, Wang et al. hypothesized that members of the upstream complex would include Get4 and Get5, whose functions were previously shown to be required for TA insertion into the ER membrane. Even more enticing, the genetic interactions exhibited by GET3, GET4, and GET5 resembled one another (Jonikas et al., 2009). By examining early events during the *in vitro* translation of a yeast TA protein, the authors discovered that Get4 and Get5, along with a chaperone recruiting protein, Sgt2 (Liou et al., 2007), act upstream of Get3 (Fig. 1C). Mutation analysis indicated that Sgt2 binds Get5, which in turn is tethered to Get4. Hsp104 and Hsp70 chaperones, along with an uncharacterized cytoplasmic protein, were also members of this complex and were linked to Sgt2 through a TPR domain. More importantly, Sgt2 directly binds a TA sequence via a Met-rich sequence. By analogy, a Met-rich domain in an SRP subunit serves as the signal sequence recognition motif. Future structural studies should enlighten us as to whether the Sgt2-mediated recognition of a C-terminal TA sequence is similar to the SRP-mediated binding of an N-terminal signal sequence.

Given the direct binding of Sgt2 to a TA sequence, it was reasonable for Wang et al. to next ask whether Sgt2 mediates the decision between ER and mitochondrial targeting. Consistent with this hypothesis, a mitochondrial-targeted TA protein interacted poorly with Sgt2 and inserted inefficiently into ER-derived microsomes. In contrast, an ER-targeted, mutated version of the same TA protein associated ~10-fold better with Sgt2, and microsome insertion mirrored the improvement in Sgt2 association. The authors then found that TA protein insertion could be reconstituted with Sgt2 and Get3-Get4-Get5. This system was co-opted to show that Get4-Get5 facilitates the interaction between Get3 and the Sgt2-TA protein complex, and between a Get3-Sgt2-TA protein complex and the membrane.

Overall, the results in this paper have defined critical, early events during TA protein biogenesis, and established that Sgt2 aids in the selection of ER-targeted TA proteins. How then is mitochondrial targeting dictated? By examining the activity of a TPR domain mutant form of Sgt2, the authors proposed that an Sgt2-associated chaperone, perhaps Hsp70, selects mitochondrial TA proteins. At first, this seems at odds with data indicating that some mammalian TA proteins targeted to the ER utilize the Hsp70 machinery (Rabu et al., 2008). However, it is possible that while the Hsp70 associated with Sgt2 functions during mitochondrial targeting, the cytoplasmic, free pool of Hsp70 aids in the TA insertion of select ER targeted proteins; this pool would be unaffected by the mutation in Sgt2's TPR domain. It is also intriguing that Hsp104, a hexameric AAA-ATPase with disaggregase activity, is intimately connected to the TA insertion machinery. Because mis-targeted TA proteins can aggregate (Schuldiner et al., 2008; Jonikas et al., 2009), positioning Hsp104 near nascent TA proteins may lessen the propensity of these substrates to wreak havoc if targeting is delayed or is inefficient. If so, in higher eukaryotes that lack Hsp104, does another chaperone function in its place?

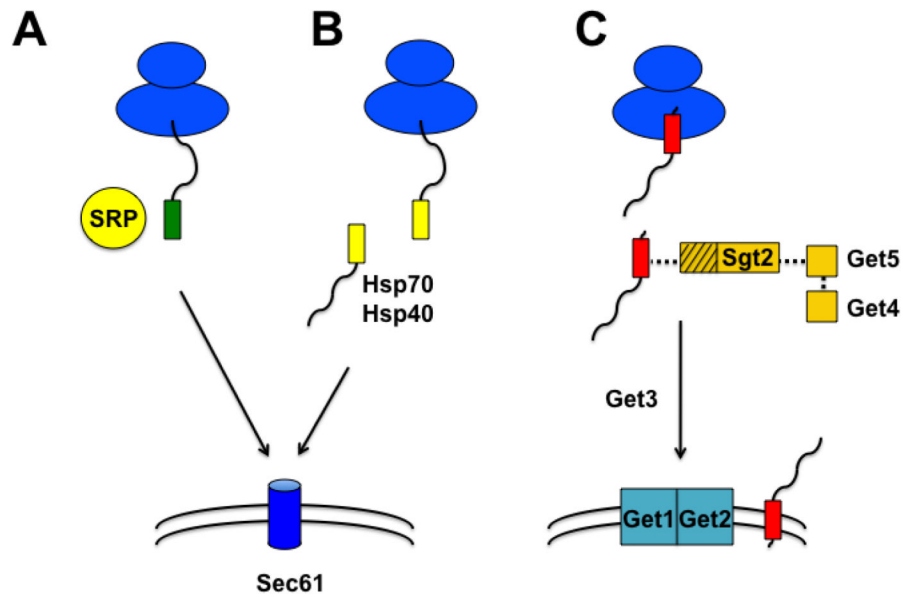
Finally, the data in this paper suggest that the Get4-Get5-Sgt2 complex may recognize TA proteins at the ribosome. Recently, the Hegde lab reconstituted early events during the delivery of a TA protein to the mammalian ER (Mariappan et al., 2010). In line with the data reported in Wang et al., a TA-binding protein was identified within a stable complex that

functions upstream of TRC40. The components of the complex are Bat3 (also known as Bag6/Scythe), TRC35 and Ubl4A. TRC35 and Ubl4A, respectively, are homologous to Get4 and Get5. Surprisingly, Bat3 and Sgt2 are not homologous. Why is one of the two mammalian Sgt2 homologs absent from this complex? Have different factors (i.e., Bat3) evolved as specificity determinants for distinct classes of TA proteins? The evolution of a greater and more diverse number of TA proteins in mammals might have been accompanied by an expanded repertoire of TA-binding chaperones. For example, some TA proteins may need to be recognized at the ribosome, whereas others may encounter their chaperone partners after ribosome release. Notably, Mariappan et al. measured a >5-fold enrichment of the Bat3 complex on ribosomes that were synthesizing a TA protein. This result suggested that the Bat3 complex must be recruited to the ribosome within a narrow time frame, namely, as the TA sequence is synthesized. To increase its chances to bind a nascent TA sequence, the authors discovered that the Bat3 complex has stolen a page from the SRP playbook: the complex slows translation and thus elongates the time frame during which it can interact with the TA protein.

Overall, the recent papers from the Hegde and Denic laboratories provide complementary insights into the TA protein insertion pathway in the yeast and mammalian systems. They also highlight notable gaps in our understanding of TA protein biogenesis. Nevertheless, these exciting results dictate that it may soon be time to rewrite the cell biology textbooks to include the mechanisms of action of the Sgt2 and Bat3 complexes.

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**Figure.**

The co- and post-translational translocation pathways in yeast. Proteins can be targeted to the ER membrane either (A) co-translationally by SRP, or (B, C) post-translationally.

(A) SRP interacts with an N-terminal signal sequence (in green) and slows translation, favoring the interaction of the nascent chain with the Sec61-containing translocation machinery.

(B) Alternately, N-terminal signal sequences (yellow) can direct the Hsp70-Hsp40-mediated translocation of post-translationally translocated substrates, which also utilize Sec61.

(C) Proteins with C-terminal, transmembrane TA sequences (red) are recognized by the Sgt2-Get4-Get5 complex through a TA-binding domain in Sgt2 (depicted by diagonal lines). The TA protein is then handed off to Get3, an event that is facilitated by the Get4-Get5 complex. Next, Get3 may undergo an ATP-dependent conformation change to deliver the substrate to the Get1-Get2 complex in the ER membrane, which is also facilitated by the Get4-Get5 complex. The mechanism by which the TA protein is ultimately inserted into the lipid bilayer is unclear. The association of Sgt2 with Hsp104, Hsp70, and Ybr137w is not shown.