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The Tail End of Membrane Insertion

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Many membrane proteins are inserted into cellular membranes via a carboxy-terminal tail-anchor segment, but the mechanism of insertion is poorly understood. In this issue of *Cell*, Stefanovic and Hegde (2007) report the identification and initial characterization of a soluble ATP-dependent receptor for the insertion of newly synthesized tail-anchored membrane proteins.

An important class of proteins in eukaryotic cells are tail-anchored (TA) membrane proteins, which include cytochrome b_5 (the founding member of the TA family), the SNARE proteins involved in vesicle trafficking, as well as proteins involved in apoptosis (Bcl-2 family), and several subunits of the mitochondrial and endoplasmic reticulum (ER) protein translocation channels. The yeast genome encodes 55 tail-anchored membrane proteins (Beilharz et al., 2003) that are ultimately localized to the nuclear envelope, the outer mitochondrial membrane, the peroxisome, and all membranes within the exocytic and endocytic pathways. Despite their physiological significance, the mechanism responsible for insertion of most TA proteins into membranes has remained elusive. Most membrane proteins have N-terminal transmembrane segments or cleavable signal sequences and are cotranslationally targeted to the ER by the signal recognition particle (SRP) and integrated into the membrane via the Sec61 translocon pore (Figure 1). However, TA proteins are integrated into membranes via a carboxy-terminal hydrophobic segment. This segment serves both as a transmembrane-spanning domain and the targeting signal for initial insertion of the protein into either the ER or outer mitochondrial membrane. The C-terminal location of the tail-anchor segment precludes cotranslational recognition of the transmembrane-spanning region by SRP. Cytochrome b_5 can spontaneously insert into phospholipid vesicles (Kim et al., 1997) and its integration into membranes occurs independent of SRP, Sec61, and ATP hydrolysis (Anderson et al., 1983; Yabal et al., 2003; Kim et al., 1997). Yet, spontaneous insertion appears to be more the exception than the rule for TA membrane proteins that are integrated into the ER. Membrane insertion of the exocytic SNARE protein synaptobrevin requires ATP hydrolysis and one or more protease-sensitive ER membrane proteins (Kutay et al., 1995)

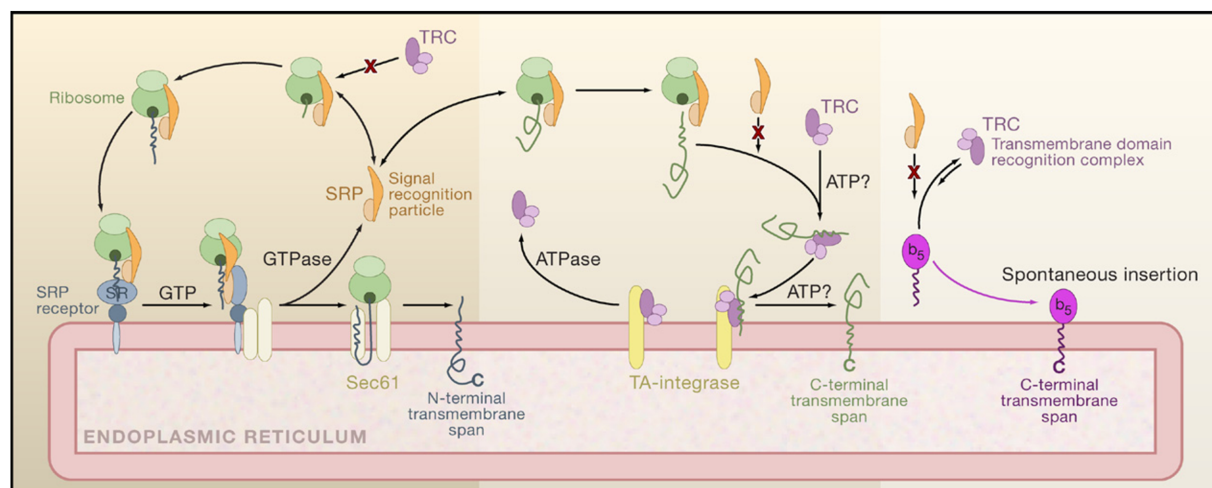

Figure 1. Cotranslational and Posttranslational Integration of Membrane Proteins
Partitioning of newly synthesized membrane proteins between the SRP-dependent cotranslational pathway and the TRC-dependent posttranslational pathway is determined by the location of the transmembrane span (N- or C-terminal) and the interaction between SRP and the ribosome. A subset of tail-anchored (TA) proteins (e.g., cytochrome b_5) can integrate in a TRC- and TA-integrase-independent reaction. Nucleotide binding and hydrolysis reactions regulate assembly and disassembly of complexes between targeting receptors and membrane components.

but, importantly, not the SRP receptor or the Sec61 translocon. In *Saccharomyces cerevisiae*, insertion of TA proteins into microsomes (ER-derived vesicles) is not dependent upon the cotranslational protein translocation channel (Sec61 complex), an auxiliary translocation channel (Ssh1 complex) or the SEC complex that mediates posttranslational translocation (Steel et al., 2002). Although these studies provided strong evidence that typical TA proteins are integrated by a novel protein-mediated ATP-dependent pathway, the lack of viable candidates for a targeting receptor and an integrase for TA proteins has prompted other investigators to re-explore the involvement of the core translocation machinery (SRP, SRP receptor, and the Sec61 complex) in TA membrane protein integration (Abell et al., 2004).

With the identification of TRC40 as the core subunit of a soluble *t*ransmembrane domain *r*ecognition *c*omplex (TRC), Stefanovic and Hegde (2007) have eloquently answered several major questions concerning the mechanism of TA-protein insertion. Using an improved in vitro assay to detect integration of the model TA protein Sec61β, the authors demonstrate that Sec61β integration is neither spontaneous nor dependent upon the core translocation machinery. Protein crosslinking experiments revealed that newly synthesized Sec61β binds to a soluble 40 kDa protein in a TA-segment-dependent manner. Immunopurification of this complex followed by mass spectrometry led to the identification of TRC40. Mammalian TRC40 had been previously characterized as Asna1, an ATPase that is 27% homologous to a prokaryotic protein that regulates arsenite transport. A homozygous knockout of the mouse Asna1 gene causes embryonic lethality (Mukhopadhyay et al., 2006). Additional evidence that the TRC-Sec61β complex is a bona fide intermediate in the TA protein insertion pathway was provided by the following observations: (1) crosslinks between TRC40 and Sec61β, which represent an intermediate in the insertion pathway, are drastically reduced by the addition of microsomes (into which Sec61β gets inserted) but not by liposomes; (2) a dominant-negative (i.e., ATPase-defective) TRC40 mutant binds to, but does not release, nascent Sec61β when microsomes are present; (3) wild-type TRC, which exists in a soluble and microsome-bound form, is partially extracted from the ER by ATP. Taken together, these results suggest that TRC is the long-sought-after targeting receptor for TA-protein insertion. Targeting of TRC to a membrane-bound TA-protein integrase is proposed to precede ATP-hydrolysis-dependent transfer of the nascent TA protein to the integrase (Figure 1).

A Sec61β-GFP, but not a GFP-Sec61β, chimera is integrated by the cotranslational pathway (Stefanovic and Hegde, 2007), thereby showing that the C-terminal location of the targeting signal is critical for directing TA-anchor proteins into the posttranslational TRC-dependent targeting pathway. Lack of an interaction between TRC and the ribosome supports the conclusion that SRP and TRC do not compete for substrates due to the nonoverlapping location of the targeting sequence (N-terminal versus C-terminal) and due to the temporally and spatially restricted nature of cotranslational SRP recognition (Figure 1).

The discovery of TRC40 opens up multiple avenues of investigation concerning TA protein biogenesis. Immunopurified TRC, which sediments as a 200 kDa complex on sucrose density gradients, has additional subunits of unknown identity and function. These TRC subunits could regulate the TRC40 ATPase cycle or mediate interactions with the TA-protein integrase. Identification and purification of the TA integrase will allow a mechanistic analysis of TA-protein insertion into proteoliposomes using purified components.

Given the essential cellular function of many yeast TA membrane proteins, it is surprising that the yeast ortholog of TRC40 (Get3p) is encoded by a nonessential, nonredundant gene. However, yeast *get3*Δ mutants show pleiotropic phenotypes that may well be explained by a reduced capacity to insert TA membrane proteins. Clearly, the viable phenotype of the *get3*Δ strain necessitates the existence of a bypass pathway for TA-protein insertion in yeast, which could be the posttranslational translocation pathway mediated by the SEC complex. Yeast Get3, which forms complexes with two integral membrane proteins (Get1 and Get2), has been implicated in retrograde vesicular traffic between the Golgi and ER (Schuldiner et al., 2005). Although Get3 (TRC40) may have multiple cellular roles depending upon its interacting partners, the possibility that Get1 and Get2 are subunits of the TA integrase should be explored.

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