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PROTEIN TRANSLOCATION

A tale of topology

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Many membrane proteins are targeted to biological membranes by an N-terminal signal sequence or transmembrane domain (TMD) that is recognized by the translocation machinery during translation — the co-translational pathway. However, in tail-anchored (TA) membrane proteins — such as SNARE proteins and members of the BCL-2 family — the only targeting information is encoded in a single TMD close to the C terminus. Stefanovic and Hegde now identify the first component of the complex that targets TA proteins for membrane insertion.

TA proteins cannot use the co-translational pathway because the protein is released from the ribosome before the C-terminal TMD emerges from the ribosomal tunnel. Instead, TA proteins are post-translationally inserted into membranes by a poorly defined mechanism. Using cross-linking studies to identify proteins that interact with TA proteins, Stefanovic and Hegde identified the TMD recognition complex-40 (TRC40) protein, which robustly binds to TMDs of TA proteins. TRC40 cross-linked to other unidentified proteins, which suggests that it is part of a larger complex. TRC40 interacted with TA proteins in the cytosol before, but not after, membrane insertion. Therefore, the TRC appears to bind free TA proteins, and perhaps aids their delivery to membranes.

TRC40 has ATPase activity and was ubiquitously expressed in all tissues examined. An ATPase-deficient mutant of TRC40 dominantly inhibited TA-protein insertion but had no effect on non-TA proteins. To investigate the basis of this selectivity, the authors constructed variants of the model TA protein Sec61β with a large tag added at either the N or the C terminus. Remarkably, the interaction with TRC40 was lost selectively for the C-terminally tagged Sec61β, the TMD of which was no longer at the end of the protein. Therefore, the cue for recognition by TRC40 is not simply the presence of a TMD; instead, the TMD must reside in a suitable context near the C terminus of the protein, a defining feature of TA proteins.

TRC40 binds to isolated endoplasmic reticulum (ER) membranes, which suggests the presence of TRC40 receptors in the ER. This starts to parallel the co-translational pathway in which the N-terminal signal sequence is bound by a cytosolic signal-recognition particle (SRP) that, through binding to ER-localized SRP receptors, delivers the substrate to the ER protein translocon. However, the two pathways do not share components and other differences are already apparent; for example, the release of TRC40 from ER membranes is stimulated by ATP, whereas the release of SRP is dependent on GTP.

TRC40 was previously identified as ASNA1, and deletion of *Asna1* in mice causes early embryonic lethality. However, yeast lacking the TRC40 orthologue, Get3, show pleiotropic defects in various membrane-associated processes; their viability hints at redundant pathways for TA-protein insertion in this organism. Proteins that interact with Get3 as part of the Get complex in yeast could represent other components of the TA membrane-protein insertion pathway. However, proteins that are homologous to Get1 or Get2 in other organisms have yet to be identified.

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ORIGINAL RESEARCH PAPER Stefanovic, S. & Hegde, R. S. Identification of a targeting factor for posttranslational membrane protein insertion into the ER. *Cell* **128**, 1147–1159 (2007)
FURTHER READING Mandon, E. C. & Gilmore, R. The tail end of membrane insertion. *Cell* **128**, 1031–1032 (2007)
WEB SITE
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