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Sommario	Tail-anchored (TA) proteins constitute a class of membrane proteins whose diverse members carry out basic functions in cell physiology, including regulation of exocytosis and of apoptosis. TA proteins share a particular topology, consisting in a cytosolically located N-terminal domain anchored to the bilayer by a C-terminal hydrophobic stretch. Because of this particular topology, these proteins are targeted to their destination by unique post-translational pathways. An important identified pathway is centred on the ATPase TRC40/Get3, however, at least under cell-free conditions, some TA proteins can access membranes independently from this pathway, and can even insert spontaneously into protein-free phospholipid bilayers without assistance from any chaperone. One example of a spontaneously inserting TA protein is cytochrome b5, a protein involved in lipid and drug metabolism. Two forms of cyt b5 are known, which in vivo target either the Endoplasmic Reticulum (b5-ER) or the mitochondrial outer membrane (b5-RR). The question addressed in my thesis is how in vivo specificity is attained in the face of in vitro promiscuity. To investigate this problem, I set up a system based on semi-intact cultured cells, which can be manipulated and analysed biochemically and by immunofluorescence. In the presence of cytosol, both b5-RR and b5-ER, added either as recombinant proteins or as in vitro

	translated products, were faithfully targeted to their correct destinations. In contrast, in the absence of cytosol, both forms targeted the mitochondria, indicating that cytosolic factors are required to avoid mislocalisation of b5-ER to the mitochondrial outer membrane. I further demonstrated that ER targeting is energy-dependent, and that both the TRC40 and the recently described SND2 pathways are minimally involved. To elucidate further pathways, I used a number of small molecule inhibitors, including ones that target heat shock proteins and the AAA-ATPase p97. Hsc70 and Hsp90 inhibition had no effect, whereas Eeyarestatin (ES I), an inhibitor of both p97 and Sec61 (the translocon responsible for co-translational translocation), strongly reduced insertion of b5-ER, but not of the TRC40 substrate Synaptobrevin 2. Similarly, I found that downregulation of Sec61, while having no effect on Synaptobrevin, inhibited b5-ER insertion, suggesting that ES I is acting by blocking the translocon. Even when Sec61 and the TRC40 pathway were blocked together, however, a large proportion of b5-ER still reached the ER, indicating the existence of yet additional pathways. My results indicate that the mitochondrial outer membrane represents the default destination of TA proteins, and reveal the existence of multiple, redundant, but substrate-specific ER targeting pathways.
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