Universität Basel

Research Project

Topogenesis and intracellular sorting of membrane proteins

Third-party funded project

Project title Topogenesis and intracellular sorting of membrane proteins
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A—Topogenesis of membrane proteins at the Sec61 translocon

Sec61/SecY translocon, the major gateway for secretion and membrane protein integration, has been structurally characterized in great detail. Yet, the mechanism and dynamics of signal sequence insertion and transmembrane integration remain challenging questions. Thermodynamic equilibration between translocon and membrane has been shown to decide between integration or translocation for each protein segment in a constant context. Our goals are to analyze topogenesis of membrane proteins by studying ...

- what influences membrane integration (stop-transfer and re-integration) besides hydrophobicity
- what drives cotranslational translocation across the membrane
- whether transmembrane hairpins facilitate topogenesis of multispanning proteins
- how translocon inhibitors (cotransin, mycolactone) prevent signal peptide insertion.

Our experimental approach is to challenge the translocon in vivo in yeast with model substrates and/or to specifically mutagenize the machineries involved.

The results will expand our understanding of membrane integration from the potential transmembrane segments themselves to the sequence context that has not previously been appreciated.

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B—Intracellular protein sorting

Protein transport in the late secretory pathway from the trans-Golgi network (TGN) to the cell surface and back is still poorly characterized. We are using/developing methods to determine anterograde and retrograde transport kinetics and to rapidly inactivate components of transport machineries in mammalian cell culture. We plan to ...

- systematically determine the kinetics of TGN-to-cell surface transport for different protein classes
- distinguish whether their pathways are direct or via endosomes
- analyze the effect of cargo size for coiled-coil proteins of different lengths
- characterize retrograde transport of a variety of membrane proteins using a novel nanobody toolkit
- quantify the effect of rapid depletion of potential transport machineries (AP-1, clathrin, Arf1/3, retromer) on anterograde and retrograde transport kinetics

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Peptide hormones self-aggregate at the TGN into secretory granules as functional amyloids for regulated secretion. We have shown that this ability to form amyloid-like aggregates is the cause of aggregation of

folding deficient provasopressin in the endoplasmic reticulum (ER) causing diabetes insipdus. We plan to test whether small disulfide loops similar to that in vasopressin is a general aggregation motif of many peptide prohormones, by testing whether they are necessary and/or sufficient for aggregation in the ER and for granule sorting in the TGN.

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In addition, we will follow up on our discovery that Rabaptin5, an early endosome regulator, interacts with FIP200, a component of the ULK1/ATG13 autophagy initiator complex. We plan to test the hypothesis that Rabaptin5 recruits the autophagy machinery to damaged endosomes and Salmonella containing vacuoles. We will analyze the molecular interaction with FIP200 and the contribution of other endosomal proteins

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The results are expected to provide novel mechanistic insights into fundamental cellular processes in protein transport, in some parts also with potential implications for disease.

Keywords Translocon, Sec61, nanobody, retrograde transport, rabaptin5, vasopressin, secretory granules
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Follow-up project of 3243488 Topogenesis and intracellular sorting of membrane proteins

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