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Irmgard Sinning

Molecular Machines in protein targeting and membrane protein biogenesis

Goal

To understand the structure and function of molecular machines in co- and post-translational protein targeting.

Background

Membrane proteins comprise more than 25% of the cellular proteome and their function depends on insertion into the correct target membrane. Membrane proteins utilize predominantly the universally conserved co-translational delivery pathway of the signal recognition particle (SRP). This pathway elegantly couples protein synthesis at the ribosome to membrane targeting and insertion, and avoids exposure of hydrophobic transmembrane domains. Although the composition of the SRP system differs in the three kingdoms of life, the central SRP core consisting of SRP54 and its binding site on the SRP RNA are conserved. SRP recognizes signal sequences at the N-terminus of newly synthesized polypeptides in the context of a translating ribosome (Fig. 1). Subsequent interaction of SRP with the membrane bound SRP receptor (SR) involves the formation of a symmetric hetero dimer of the two GTPases present in SRP and SR, which directs the ribosome nascent chain (RNC)/SRP complex to the ER in eukaryotes and to the plasma membrane in bacteria. We study the molecular mechanisms of how SRP and SR participate in protein targeting by a combination of biochemical techniques and X-ray crystallography as our key method. Our data provide structural snapshots of SRP and SR in distinct functional states that are combined into a movie of SRP driven membrane protein biogenesis. In particular, we are interested in the role of membrane lipids in the regulation of SR activity and in the molecular mechanisms of SRP GTPases.

In contrast to the SRP system, post-translational targeting delivers proteins when their synthesis is already completed. Tail-anchored (TA) membrane proteins contain a single transmembrane domain at their C-terminus which excludes them from the co-translational pathway (Fig. 1). They play important roles in membrane insertion, membrane fusion and apoptosis. Recently, the so-called Get (**g**uided-**e**ntry of **t**ail-anchored membrane proteins) pathway has been discovered that delivers TA proteins to the ER. Like other post-translational targeting pathways, the Get pathway depends on ATP. We started a detailed comparative analysis of the SRP and Get pathways in order to unravel mechanistic details and common principles of regulation.

Although the SRP system is conserved in evolution, it can be adapted for specific requirements. The post-translational function of SRP in chloroplasts is particularly interesting as it guides nuclear encoded light-harvesting chlorophyll *a,b* binding proteins (LHCPs) to the thylakoid membrane. LHCPs serve as antenna complexes in photosynthesis and are the most abundant membrane proteins on our planet. They contain three hydrophobic transmembrane helices and have to kept in a conformation competent for membrane insertion. We study the structure and function of cpSRP43, a novel component of cpSRP, in order to understand its role in LHCP biogenesis.

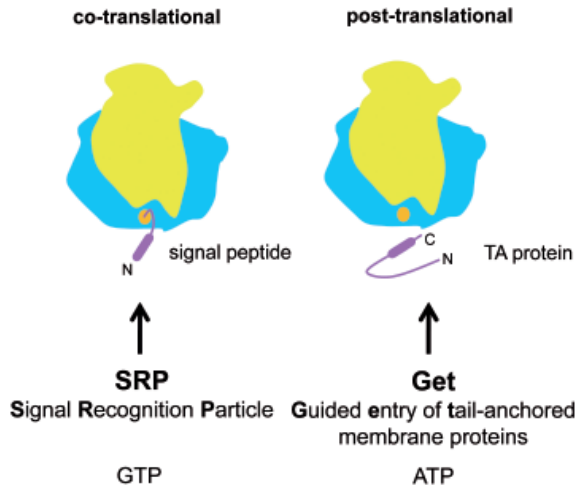

Fig. 1: Recognition of targeting signals by SRP and Get pathways.

Research Highlights

cpSRP43 is characterized by a unique arrangement of chromodomains and ankyrin repeats. Our crystal structure of cpSRP43 revealed that it resembles the SRP RNA (Fig. 2). While chromodomains are almost exclusively known for their key role in the regulation of gene expression, reading the so-called histone code, ankyrin repeats are well established as versatile protein interaction modules. In cpSRP43 the ankyrin repeats provide the binding site for an internal signal sequence present in LHCPs, the L18 region (Fig. 2). Moreover, we could show that a 'DPLG' motif within L18 is required to recruit LHCPs into a soluble transit complex that enables LHCP delivery to and insertion into the thylakoids. cpSRP43 is therefore more than an adaptor that allows to highjack the conserved SRP system for post-translational protein targeting. It recognizes its membrane protein cargo in a most SRP-unlike manner – with high sequence specificity. We could show that cpSRP43 acts as a chaperone for membrane proteins and localize the primary chaperone function to the ankyrin repeats. In contrast, most chaperones are large proteins or assemblies that require ATP hydrolysis for their function. We discovered that cpSRP43 can even act as a disaggregase that is able to dissolve LHCP aggregates without ATP hydrolysis. We also clarified the role of the two C-terminal chromodomains of cpSRP43.

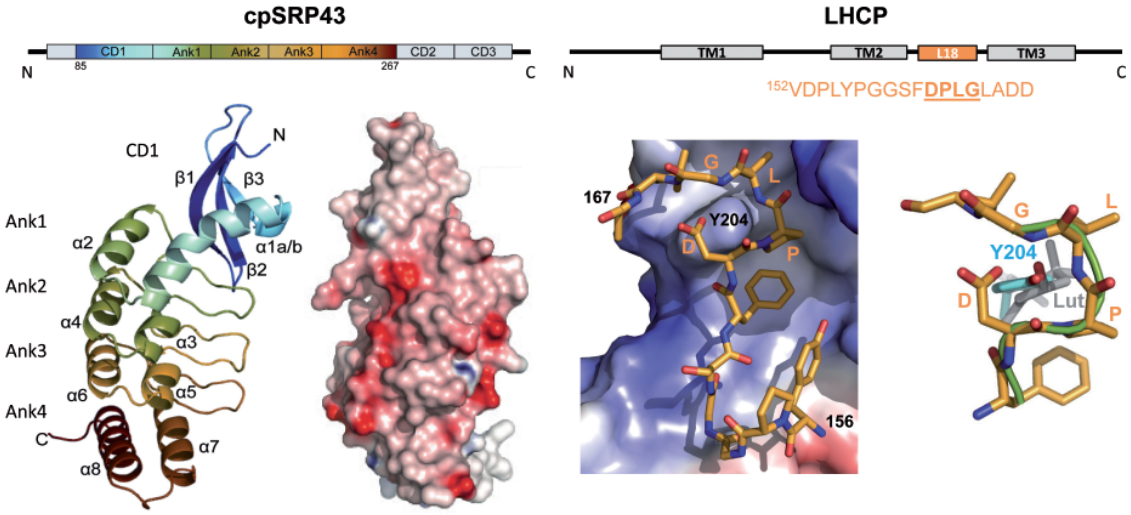

Fig. 2: cpSRP43 serves as a specific membrane protein chaperone. *cpSRP43 consists of ankyrin repeats and chromodomains (left). Ank1-4 specifically bind a 'DPLG' motif within LHCPs (right) and keep it in a conformation competent for carotenoid (lutein) attachment upon membrane insertion.*

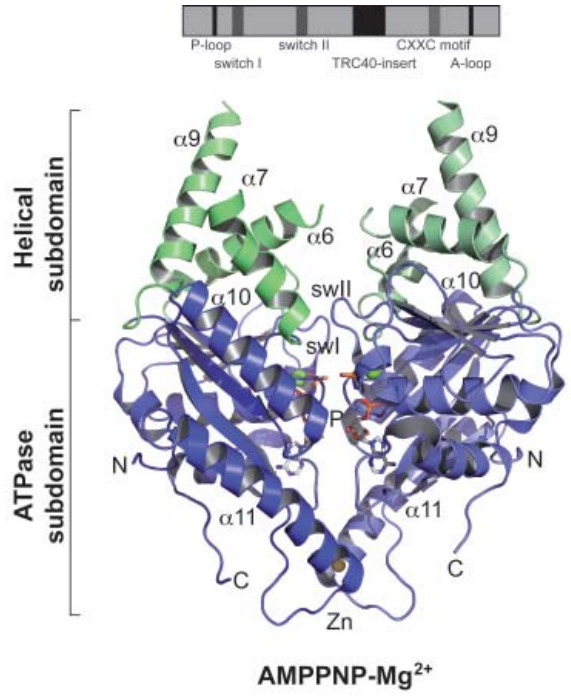

Fig. 3: Structure of Get3. *The ATPase forms a dimer clamped together by a Zn ion and comprises two subdomains (blue, green).*

They are involved in the interaction with cpSRP54 and with the chloroplast member of the YidC/Oxa1/Alb3 family of the membrane insertases. The C-terminal tail of Alb3 contains two motifs enriched in positive charges that are required to bind cpSRP43. Our studies suggest a model for LHCP delivery to the thylakoid membrane. In order to understand how Alb3 and its homologs act as membrane insertases we continue our efforts towards the structure determination of Alb3 and homologs.

The SRP GTPases form a distinct subfamily of the SIMIBI (for Signal Recognition Particle, MinD, BioD) class of NTP binding proteins with only three members: the SRP core protein SRP54, the SRP receptor protein FtsY (in bacteria; SRα in eukaryotes) and FlhF, a protein involved in the assembly of polar flagella. We have previously identified a conserved membrane targeting sequence (MTS) in FtsY that is required and sufficient for directing the SRP receptor to the plasma membrane. Combining amide hydrogen-deuterium exchange with mass spectrometry (HX-MS), X-ray crystallography and CD spectrometry we could now show that the interaction with anionic phospholipids triggers a conformational switch of the MTS. This switch allows for subsequent activation of the FtsY GTPase which is crucial for SRP mediated protein targeting.

The central component of TA membrane protein biosynthesis, the ATPase Get3, is also a member of the SIMIBI class of NTP binding proteins. Structure determination of Get3 in different nucleotide loaded states (Fig. 3) together with membrane insertion assays (with B. Dobberstein, ZMBH) allowed us to propose a model for how the ATPase cycle of Get3 is linked to TA protein binding and release. HX-MS was used to localize the TA protein binding site in Get3 to a hydrophobic subdomain formed by two insertions in the ATPase fold. Interestingly, the TA protein binding site shares the enrichment in methionine residues with the signal sequence binding site in the M domain of SRP54. Although the co- and posttranslational functions of the SRP and Get pathways differ, the basic principles of cargo recognition are conserved.

We continued **additional research activities** in small teams: **Gert Bange** studies flagella biosynthesis in *Bacillus*. Flagella are one of nature's largest molecular machines and act also as virulence factors - besides their role in locomotion. The translocation of flagella building blocks involves a type III secretion system (TTSS) which comprises a number of membrane proteins. FlhA is the largest component of the TTSS and the structure of its cytosolic domain provided first insights into the domain architecture (Fig. 4). Together with biochemical data, we clarified the role of chaperones in the coordinated delivery of late flagellar building blocks to the TTSS. **Valerie Panneels** optimizes a novel expression system for membrane proteins developed previously in our lab. It exploits the naturally abundant membrane stacks in the photoreceptor cells (PRCs) in the eyes of *Drosophila melanogaster*. We analysed how endogeneous rhodopsin is

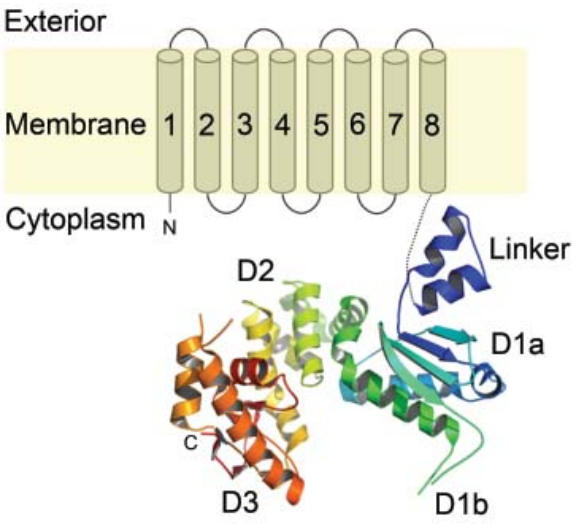

Fig. 4: Domain arrangement of the FlhA cytosolic domain.

targeted to the rhabdomeres. We could localize the targeting signal of Drosophila rhodopsin in the distal part of helix 8 which might be a useful tool to improve heterologous expression of GPCRs and transporters. Several receptors and transporters produced in fly eyes have entered into crystallization trials. **Ivo Tews** studies the molecular mechanisms of Toc GTPases in chloroplast import, mycobacterial adenylylcyclases and Vitamin B6 biosynthesis. Structural studies of PLP synthase provided insights into the assembly mechanism of this huge molecular machine and highlighted a number of key intermediates in PLP synthesis. Chloroplasts contain a majority of proteins that are nuclear encoded, synthesized in the cytosol and imported into the stroma across the outer and inner envelope. Import is regulated by the GTPases Toc33 and Toc159. Structure analyses and biochemical data clarified the role of Toc33 dimerization for protein import. **Klemens Wild** analyses the structure and function of amyloid precursor protein (APP) complexes. APP is the central player in Alzheimer Disease pathogenesis. Structures were determined of the APP intracellular domain (AICD) in complex with a physiologically and pathologically important phosphotyrosine-binding domain Fe65-PTB2 and of the Fe65-PTB1 domain, which constitutes a main crossroad in APP signaling and trafficking.

Selected Publications 2008 - 2010

Falk, S. & Sinning I. (2010) cpSRP43 is a novel chaperone specific for light-harvesting chlorophyll a,b binding proteins, J. Biol. Chem. 285: 21655-61.

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Wild, K., Bange, G., Bozkurt, G., Segnitz, B., Hendricks, A. & Sinning, I. (2010) Structural insights in RNP assembly of the human and archaeal signal recognition particle, Acta Cryst. D 66: 295-303.

Falk, S., Ravaud, S., Koch, J. & Sinning, I. (2010) The C-terminus of the Alb3 membrane insertase recruits cpSRP43 to the thylakoid membrane, J. Biol. Chem. 285: 5954-62.

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Ravaud, S., Stjepanovic, G., Wild, K. & Sinning, I. (2008) The crystal structure of the periplasmic domain of the *Escherichia coli* membrane protein insertase YidC contains a substrate binding cleft, J. Biol. Chem. 283: 9350-8.

Awards and Honors

2010 Member of EMBO

2010 Member of LEOPOLDINA

2010 Heidelberg Molecular Life Sciences (HMLS) Investigator Award

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