1989	Ph.D. - Ludwig-Maximilians-Universität München, Germany (Max Planck Institute of Biochemistry, Martinsried)
1989 - 1991	PostDoc - Max Planck Institute for Biophysics, Frankfurt, Germany (Prof. Hartmut Michel)
1991 - 1993	Scientist - Biomedical Centre, Uppsala, Sweden (Prof. Alwyn Jones)
1994 - 1999	Group Leader - European Molecular Biology Laboratory (EMBL), Heidelberg, Structural Biology Programme
since 2000	Full Professor - BZH
2006 - 2009	Director - BZH
2011 - 2013	President of the GBM

Irmgard Sinning

Macromolecular machines in protein targeting and membrane protein biogenesis

Goal

We aim to understand the structure and the functional mechanisms of macromolecular assemblies in protein folding, targeting and membrane protein biogenesis.

Background

Already during protein synthesis at the ribosome, nascent proteins experience numerous interactions with (1) targeting factors, and (2) chaperones and enzymes (**Fig. 1**). We aim at detailed insights into the structure and function of the involved molecular machines by an integrated structural biochemistry approach combining protein crystallography as our key method with biochemical and biophysical techniques.

(1) Our long-term interest is the understanding of the delivery pathways for membrane proteins by the signal recognition particle (SRP) and the system for guided-entry of tail-anchored membrane proteins (Get). The SRP pathway directly couples protein synthesis at the ribosome to membrane targeting and insertion, thereby the exposure of hydrophobic transmembrane domains is avoided. SRP recognizes a signal sequence at the N-terminus of target proteins, while tail-anchored (TA) membrane proteins contain a single transmembrane domain at their C-terminus. This excludes Get substrates from the co-translational SRP pathway. Although the composition of the SRP system differs in the three kingdoms of life, the central SRP core consisting of SRP54 and its cognate binding site on the SRP RNA are conserved. However, the SRP system has also been adapted for specific requirements. Here the post-translationally acting SRP in chloroplasts (cpSRP), which lacks the SRP RNA, is an interesting example. It guides nuclear encoded light-harvesting proteins (LHCPs) to the thylakoid membrane assisted by cpSRP43, a unique component of cpSRP. Over the years, we collected structural snapshots of SRP and SRP receptor (SR) as well as of components of the Get machinery in different functional states with a particular interest in the membrane-associated steps. We aim to finally arrive at a molecular movie of co- and post-translational membrane protein biogenesis in all kingdoms of life. (2) During synthesis at the ribosome, proteins are subject to enzymes for modification and chaperones that assist in folding. These factors seem to share overlapping binding sites at the ribosomal surface close to the tunnel exit. In order to understand the carefully orchestrated interplay of all these different factors, we started to dissect the mechanistic details of eukaryotic ribosome associated chaperones and enzymes.

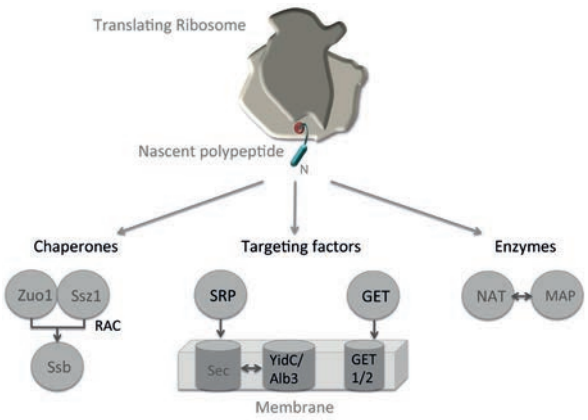

Fig. 1: *Early factors associating with the nascent polypeptide chain at the ribosome – crowding at the tunnel exit.*

Is the ribosome more than a binding platform for factors acting on the nascent chain? Are ribosomal proteins and RNA involved in the selective interaction of all these different factors with specific nascent chains? To answer these questions and to study complex macromolecular assemblies, we combine *in vitro* and *in vivo* analyses.

Research Highlights

Almost three decades after the discovery of SRP, structural and functional studies of the SRP system are rather advanced. However, important data on the bacterial, eukaryotic and chloroplast SRP systems are still missing. Eukaryotic SRP can be divided into an Alu domain involved in the retardation of protein synthesis (so-called elongation arrest) and an S domain responsible for signal sequence recognition, translocon interaction and regulation. The Alu domain is the precursor of the *Alu* elements, which represent more than 10% of the primate genome and play important roles in genome stability. In eukaryotic SRP, the Alu domain consists of half of the SRP RNA and two proteins (SRP9 and SRP14), which are essential for the Alu RNA to adopt a stable fold. In Archaea and most grampositive bacteria the Alu domain is present, but lacks the protein components. The high resolution X-ray structure of the *Bacillus subtilis* Alu RNA answered a number of important questions in the SRP field. The structure shows a conserved, highly complex tRNA-like fold with several inbuilt stabilizing elements (helix 1 and an extended loop-loop pseudoknot) and a novel mode of minor groove interactions (**Fig. 2**). It explains why proteins are dispensable for the archaeal and bacterial Alu domains, and confirmed the “closed” conformation of the Alu domain inferred from previous cryo-EM work and modeling. Placing this structure in the cryo-EM density (with R. Beckmann, Munich) allowed to deduce the mechanism of elongation retardation by competition with the aa-tRNA/EF-Tu complex at the ribosome.

Another “grey zone” in our understanding of human SRP concerned its two largest protein components, SRP68 and SRP72, which are essential for both elongation arrest and protein translocation. These proteins were notoriously difficult to handle, probably because they are highly flexible. However, we managed to first determine the

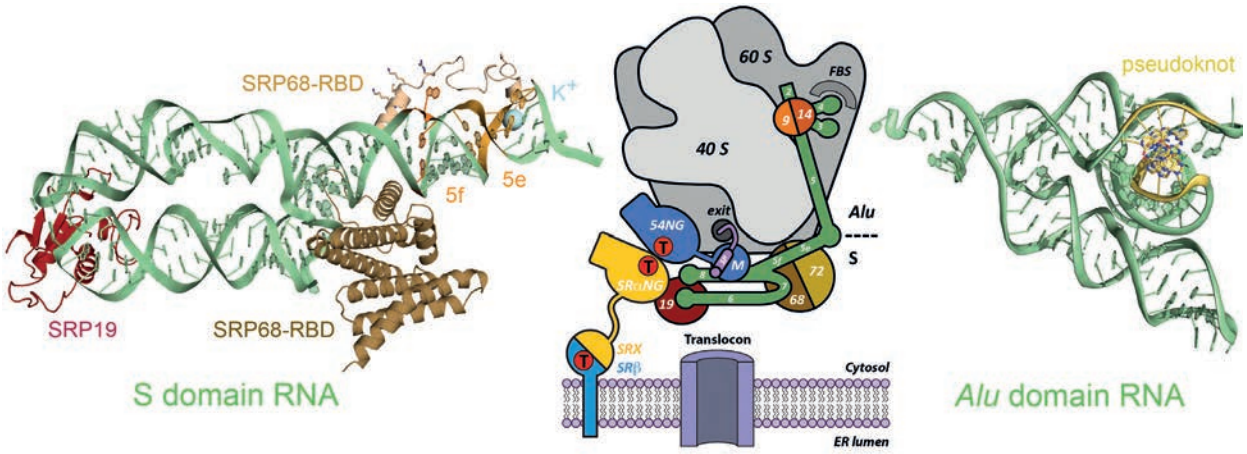

Fig. 2: Insights in to mammalian SRP (middle). Remodeling of the SRP RNA by SRP68/72 (left) *shapes the distal binding site at the 5f-loop.* ***The prokaryotic Alu domain (right)*** *lacks the protein components.*

structure of the RNA-binding domain (RBD) of SRP68 alone as well as of a large part of the human S domain (125 nts RNA, SRP19 and the 68-RBD) (**Fig. 2**). This structure provided a plethora of novel insights into protein-RNA recognition, S domain organization and SRP dynamics. SRP68 modulates the RNA structure in an unexpected way, visualizing why SRP68 is crucial for SRP function. Encouraged by this breakthrough, we continued our efforts on SRP72, and determined the structure of its protein-binding domain (interacting with the SRP68-PBD) and of the SRP72-RBD now in complex with the complete S domain RNA (145 nts RNA, SRP19, 68-RBD, 72-RBD) (**Fig. 2**). The 72-RBD appears as a linear motif that crawls along the "distal site" of the SRP RNA at the 5e- and 5f-loop, with strictly conserved residues (W577, R576, R581) stabilizing the 5e- and 5f-loop geometries. These detailed structural insights in SRP68/72 were crucial to interpret previous cryo-EM data, and allowed for a first detailed model of the human SRP targeting cycle, which involves dynamic rearrangements of SRP and its receptor at the ribosome.

The SRP GTPases form a unique subfamily of the NTP binding proteins, and during protein targeting, the GTPases of SRP54 and the SR (SRα in human SRP, FtsY in bacteria) form a quasi-symmetric heterodimer (the targeting complex, TC). With the structure determination of the archaeal, human and chloroplast TCs, we obtained detailed insights into conservation and specific adaptations of this complex, which allowed us to classify conserved binding sites for external ligands, e.g. the SRP RNA responsible for activation of GTP hydrolysis. However, the human SRP system comprises yet another G protein, SRβ, which is not an SRP GTPase and belongs to the Arf/Sar family of small G proteins. We derived the unique switch cycle of SRβ and integrated the third GTPase into the SRP pathway. Using cross-linking, ribosome binding and translocation studies (with M. Pool, Manchester) we showed that the mammalian SR contains distinct ribosome and translocon interaction sites, and that it switches the Sec61 translocase from Sec62 to SRP-dependent translocation. Thereby, we identified an important function of the SR, which mechanistically links two seemingly independent modes of translocation.

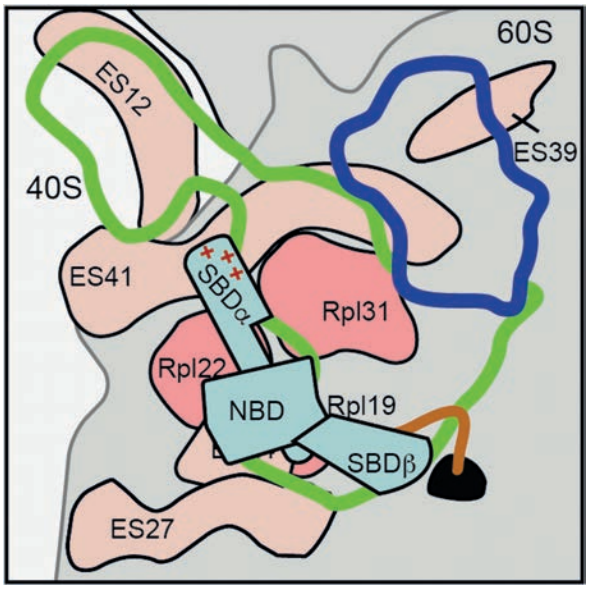

Fig. 3: Ribosome binding of the co-translational chaperone Ssb involves ribosomal proteins and RNA. *The position of RAC is outlined in blue (Ssz1) and green (Zuo1).*

While canonical membrane protein biogenesis requires the co-translational delivery of ribosome-associated proteins to the Sec translocase, the high-throughput delivery of the abundant LHCPs to the Alb3 membrane insertase in chloroplasts occurs post-translationally. Therefore, a transit complex is formed in the stroma consisting of cpSRP54, cpSRP43 and LHCPs. We have previously shown that the delivery of the transit complex to Alb3 involves positively charged motifs in the Alb3 C-terminus. Continuing our studies, we used a hybrid approach involving NMR and X-ray crystallography, and resolved the structural basis of negative cooperativity underlying cpSRP43 chromodomain interactions within the transit complex and with Alb3.

More recent research activities center on interactions of nascent chains with chaperones. The ribosome associated complex (RAC) is a unique chaperone complex, consisting of an inactive Hsp70 (Ssz1) and an Hsp40 protein (Zuo1). After resolving the interaction of RAC with the ribo-

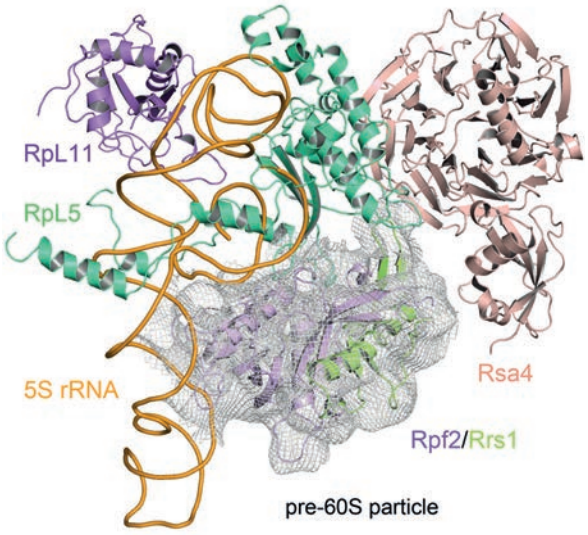

Fig. 4: Eukaryotic ribosome biogenesis. *Snapshot of 5S RNP trapped by its protein neighbours during 60S maturation.*

some by cryo-EM (with R. Beckmann), we now focused on the Hsp70 chaperone (Ssb), which is activated by RAC. Using an engineered disulfide bridge to stabilize the ATP-bound form of Ssb, we determined its crystal structure, and combined the structural information with ribosome-binding and crosslinking (with S. Rospert, Freiburg). By this integrated approach we could show that the Ssb C-terminus is required for ribosome interaction in the vicinity of the tunnel exit, and that Ssb contacts ribosomal proteins and RNA (**Fig. 3**). Our data allowed us to link the conformational rearrangements upon ATP hydrolysis to specific contacts of Ssb with the ribosome.

In the last years, we got interested in the assembly and maturation of eukaryotic ribosomes, which is a complex and highly regulated process, and involves a myriad of biogenesis factors (with Ed Hurt). We embarked on a medium throughput structural genomics project of ribosome biogenesis factors from the thermophilic fungus *Chaetomium thermophilum*, and continued on the nuclear import and assembly of the 5S RNP (consisting of 5S RNA and the two ribosomal proteins L5 and L11). Crystal structures of proteins were determined acting near the 5S RNP, including Rrs1, Rpf2 and Rsa4, which occupy strategic positions in the pre-60S ribosomal subunit to block the rotation of the 5S RNP (**Fig. 4**). Taken together, structural snapshots of ribosomal biogenesis factors are crucial to finally understand eukaryotic ribosome biogenesis.

Selected Publications 2014 - 2016

Becker, M.M.M., Lapouge, K., Segnitz, B., Wild, K. & Sinning, I. (2016) Structures of human SRP72 reveal its function in co-translational protein targeting, Nucl. Acids Res., doi: 10.1093/nar/gkw1124.

Gumiero, A., Conz, C., Valentín Gesé, G., Zhang, Y., Weyer, F. A., Lapouge, K., Kappes, J., von Plehwe, U., Schermann, G., Fitzke, E., Wölfle, T., Fischer, T., Rospert, S. & Sinning, I. (2016) Interaction of the cotranslational Hsp70 Ssb with ribosomal proteins and rRNA depends on its lid domain, Nat. Comms. 7: 13563.

Horn, A., Hennig, J., Ahmed, J.L., Stier, G., Wild, K., Sattler, M. & Sinning, I. (2015) Structural basis for cpSRP43 chromodomain selectivity and dynamics in Alb3 insertase interaction, Nat Comms. 6: 8875.

Jadhav, B., McKenna, M., Johnson, N., High, S., Sinning, I. & Pool, M.R. (2015) Mammalian SRP receptor switches the Sec61 translocase from Sec62 to SRP-dependent translocation, Nat Comms. 6: 10133.

Kharde, S., Calviño, F.R., Gumiero, A., Wild, K. & Sinning, I. (2015) The structure of Rpf2-Rrs1 explains its role in ribosome biogenesis, Nucl. Acids Res. 43: 7083-95.

Calviño, F.R., Kharde, S., Ori, A., Hendricks, A., Wild, K., Kressler, D., Bange, G., Hurt, E., Beck, M. & Sinning, I. (2015) Syo1 chaperones 5S-RNP assembly during ribosome biogenesis by RNA mimicry, Nat. Comms. 6: 6510.

Bassler, J., Paternoga, H., Holdermann, I., Thoms, M., Grannemann, S., Barrio-Garcia, C., Nyarko, A., Stier, G., Clark, S., Schraivogel, D., Kallas, M., Beckmann, R., Tollervey, D., Barbar, E., Sinning, I. & Hurt, E. (2014) A network of assembly factors is involved in remodeling rRNA elements during preribosome maturation, J. Cell Biol. 207: 481-498.

Kempf, G., Wild, K. & Sinning, I. (2014) Structure of the complete bacterial SRP Alu domain, Nucl. Acids Res. 42: 12284-94.

Grotwinkel, J.T., Wild, K., Segnitz, B. & Sinning, I. (2014) SRP RNA remodeling by SRP68 explains its role in protein translocation, Science 344: 101-104.

Leidig, C., Thoms, M., Holdermann, I., Bradatsch, B., Berninghausen, O., Bange, G., Sinning, I., Hurt, E. & Beckmann, R. (2014) 60S ribosome biogenesis requires rotation of the 5S ribonucleoprotein particle, Nat. Comms. 5: 3491.

Awards and Honors

2016	Member of the Heidelberg Academy of Sciences and Humanities
2014	Heidelberg University Research Award
2014	Gottfried Wilhelm Leibniz Prize
2014	Constitution Medal of Bavaria
2010	HMLS Investigator Award
2010	Member of EMBO
2010	Member of LEOPOLDINA

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