In all cells, proteins have to reach their final destination with high precision and specialized transport systems ensure that mis-targeting is avoided. A large variety of secretory and membrane proteins use the signal recognition particle (SRP) for their transport. We study the molecular mechanisms of how the SRP system participates in protein transport. In addition, after their insertion into membranes, we study how membrane transporters and G protein coupled receptors (GPCRs) function in membrane transport and signalling. In order to obtain a complete picture of the dynamic changes of these proteins and complexes we combine X-ray structure analysis with biochemical and biophysical methods.

**Protein transport by SRP**

Protein transport to or across the plasma membrane in bacteria and the endoplasmic reticulum (ER) in eukaryotes is mediated by the signal recognition particle (SRP). SRP is a universally conserved ribonucleoprotein particle. It recognizes amino-terminal signal sequences of newly synthesized polypeptides at the ribosome and the ribosome nascent chain (RNC) SRP complex is then targeted to the membrane by an interaction between SRP and its cognate receptor (SR) (Fig. 1). The SRP pathway is regulated by the concerted action of GTPases in both, the SRP and the SR.

**Structural basis for SRP function**

Although SRP mediated protein transport has been studied for the past 25 years, only a few years ago there was still no structural information on any SRP component available. In order to understand how SRP GTPases regulate protein transport and how they differ from other small GTPases we have determined several structures and analysed the nucleotide binding kinetics. We could show that the interaction of the bacterial SRP receptor with the membrane activates the GTPase through conformational changes. Together with B. Dobberstein (ZMBH) we established the role of the third GTPase in the human SRP system (SRb) in coordinating the release of the signal peptide from SRP with the insertion into the translocation pore. Again together with B. Dobberstein (ZMBH) we could show that the SRP receptor modulates the interaction of SRP
with the ribosome. Structures of sub-complexes of SRP (SRP54/RNA and SRP19/RNA) provided insight into the assembly of SRP. SRP54 acts as the adaptor between the ribosome and the translocon. SRP54 is a flexible multidomain protein and the only component which is conserved in all SRP systems. Since the three-dimensional arrangement of the SRP54 domains is the basis for communication between them, we have solved the structure of the complete SRP54 from *S. solfataricus* with and without helix 8 of the SRP RNA (Fig.2). The structure allowed us indeed to identify important contacts within SRP54. Future work will address the precise contribution of these regions for SRP function.

**Structure and function of membrane transporters and GPCRs**

For membrane proteins the structural data base is still strongly biased towards bioenergetic proteins since they are available in rather large quantities in their native membranes, such as the thylakoids of chloroplasts for the photosystems or mitochondria for protein complexes of the respiratory chain. Most of the membrane transporters or G protein coupled receptors (GPCRs) are however only present in their respective membranes in minute amounts. For many of them the large scale production of active and homogeneous protein is a major bottle neck for further structural and biochemical assays. In order to perform routine crystallization trials mg amounts of the protein are necessary. For some of the transporters and GPCRs we were interested in, the conventional expression systems did not yield sufficient protein. Therefore, we decided to develop a novel expression system.

**Expression in the eye of transgenic Drosophila melanogaster**

Photoreceptor cells in the eye of *Drosophila* as well as in mammals contain highly specialized membrane stacks in which rhodopsin is naturally abundant. In *Drosophila* these membranes are called rhabdomers and we could show that it is
possible to use the targeting and insertion machinery of the photoreceptor cells for the expression of “non-resident” GPCRs. Developmental drivers (promoters) which are activated at a certain stage of development “drive” the expression of the GPCR of our interest.

One of our “test proteins” was the metabotropic glutamate receptor (mGluR) from D. melanogaster (DmGluRA) (Fig. 4). mGluRs are involved in neurotransmission and control a wide range of functions in the central nervous system, including memory and reflex responses. mGluRs belong to the third class (class C) of the GPCR superfamily. mGluRs possess a seven transmembrane (7TM) domain and a large extracellular ligand binding domain which resembles periplasmic leucine-isoleucine-valine binding proteins (LIVBPs). Using our new expression system, we could show that the yield is higher than in conventional systems, within the range needed for structural studies, and that the protein is more homogeneous in terms of glycosylation.

DmGluRA is regulated by the assembly with lipid microdomains

Although it was noted for a number of GPCRs that cholesterol plays an important role for their ligand binding activity, there were no systematic studies. Since in parallel to Drosophila we also expressed the DmGluRA in baculovirus infected insect cell (Sf9 cells), we could analyse the influence of the membrane environment on ligand binding. Quantitative lipid analysis of the different membranes and lipid microdomains using mass-spectrometry was essential for this study (collaboration with Britta Brügger, Wieland-group). We could show that the association of the TM region of DmGluRA with lipid microdomains acts as a positive allosteric regulator for ligand binding to the extra-cellular domain. How changes in the TM domain are communicated to the ligand binding domain is still not clear. One aim is now to char-

Fig. 4: Expression of the metabotropic glutamate receptor in the eye of transgenic Drosophila
acterize the interaction with cholesterol in more detail. We also apply the new expression strategy to a larger set of membrane proteins which gave only low yields or poor quality in conventional systems.

Besides these two main activities we are interested in the structure determination of relevant proteins or protein complexes. One recent example is the Tudor domain (in collaboration with M. Sattler, EMBL).

**Publications 2001 - 2003**


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