

## **Modulation of mitochondrial protein import during (patho)physiological conditions (Johannes Herrmann)**

*Mitochondrial protein import:* For their biogenesis mitochondria import 800 - 1500 different proteins from the cytosol. Most of these proteins are synthesized as precursor proteins with N-terminal targeting signals. These presequences are recognized by receptors on the mitochondrial surface to mediate the transport of the preproteins through translocases in the outer membrane (TOM complex) and the inner membrane (TIM23 complex). Studies over the last three decades on isolated yeast mitochondria identified the components of the preprotein translocases and their basic functions during the translocation process. However, it remains largely unclear how the activity of the import machinery is regulated *in vivo* to adapt the import process to the physiological needs of cells. In this project we will specifically focus on mechanisms by which the mitochondrial preprotein import machinery is regulated.

*Project 1: Regulation of preprotein import by viral capsid proteins:* Rubella virus is a human pathogen that causes severe birth defects. Teratogenicity results from deleterious interactions between virus proteins and host cell factors, but little is known about this phenomenon. Virus infection leads to the expression of large amounts of capsid protein which binds to the Golgi membrane, the site of virus budding, and to mitochondrial membranes. In a collaboration with the laboratory of Tom Hobman we could recently show that capsid binds to isolated yeast mitochondria and thereby interferes with the import of preproteins. In this project we will address two main questions: (i) What is the mechanism by which capsid protein prevents preprotein translocation? And, (ii) what is the physiological relevance of this process, i.e. why do these viruses target mitochondria? We expect that this project will provide insights into the processes by which Rubella virus proteins can regulate mitochondrial activities to improve their propagation in human cells.

*Project 2: Regulation of preprotein import by redox processes:* The preprotein translocase of the inner membrane (TIM23 complex) contains the two central membrane-embedded subunits Tim17 and Tim23. Both proteins are highly conserved among eukaryotes and are of similar structure. Tim23 presumably forms the protein-conducting channel whereas Tim17 plays a central role in the substrate-triggered gating process of the translocation pore. The first and second transmembrane spans of Tim17 are flanked at their intermembrane space sides by sequences which are highly conserved from fungi to animals. Our previous results indicate that these regions are critical for protein translocation and in particular crucial for channel gating. Interestingly, these stretches contain two invariant cysteine residues (Fig. 1, left panel), one N terminal to the first transmembrane segment and one C-terminal to the second one. The high degree of conservation of these cysteine residues indicates their critical function, presumably in the control of the import process. In this project we will explore the molecular mechanisms by which Tim17 regulates the preprotein translocase and will particularly focus on the relevance of the conserved cysteine pair.