RTG 1830
GERMAN CANADIAN
COMPLEX MEMBRANE PROTEINS
IN CELLULAR DEVELOPMENT AND DISEASE

P8

Redox processes in complex I assembly (Jan Riemer)

Complex I assembly: Complex I of the respiratory chain of mitochondria is crucial for cellular energy production. It also is a major source for reactive oxygen species (ROS), and dysfunctions of the complex have been implicated in the pathogenesis of a variety of neurodegenerative disorders. Dysfunctions often occur as a result of an impaired assembly, but at present only little is known about the biogenesis and maintenance of Complex I in mammalian cells. Seven of its subunits are encoded by the mitochondrial genome, while the remaining 38 proteins have to be imported from the cytosol. With the projects proposed here we will characterize the function of four proteins in the assembly/maintenance of Complex I: (1) Three small subunits of Complex I that are imported via a redox-regulated mechanism, and (2) The flavoprotein FOXRED1 that is critical for Complex I function.

NDUFA8, NDUFB7 and NDUFS5: The three uncharacterized nuclear-encoded Complex I subunits **NDUFS5**, **NDUFB7** and **NDUFA8** lack typical mitochondrial import signals but instead contain four conserved cysteine residues and have thus properties of proteins that are imported in a redox-dependent manner (twin-CX_9C proteins). Such proteins are typically translocated over the outer membrane of mitochondria and then trapped in the intermembrane space (IMS) by oxidative protein folding. This process is facilitated by the oxidoreductase Mia40 and is coupled to the activity state of the respiratory chain. It is unknown how this redox pathway affects the assembly and maintenance of Complex I and whether its coupling to the activity of the respiratorychain might provide a feed-back control for respiratory chain assembly.

Neither NDUFS5, NDUFB7 nor NDUFA8 have been linked to Complex I deficiencies, and they do not belong to the conserved core subunits that fulfill the basic functions electron transfer and proton pumping. Instead these twin-CX_9C proteins might fulfill functions in stabilizing the complex, preventing the generation of ROS, protecting the complex from oxidative damage, or regulating Complex I function. We will study the import and the function of these proteins as well as the role of Mia40 in Complex I biogenesis and maintenance on the molecular level. (1) We will employ a novel *in vivo* import assay that we recently developed in our group. This assay allows us to follow the kinetics of the redox-dependent import of proteins in living cells. (2) We will assess the relevance of these proteins for Complex I function by knockdown of Mia40 or either of the three twin-CX_9C proteins, and overexpression of a newly identified dominant-negative mutant of Mia40. We will then follow the fate of *in vivo* translated mitochondrial proteins (pulse-chase approach in the presence of the inhibitor of cytosolic translation emetin combined with SDS-PAGE or BN-PAGE analyses of respiratory chain complexes, respectively). In addition, we will analyze the composition, biogenesis/assembly and stability of Complex I by applying pulse-chase approaches followed by native immunoprecipitation of the whole complex.

FOXRED1: The above introduced approaches will also aid in a second project directed at understanding the biogenesis of Complex I. In this project we will detail the function of the

mitochondrial flavoprotein **FOXRED1**. Homozygous mutations in this protein have been linked to isolated complex I deficiencies. The cells of these patients expressed FOXRED1 mutants, and the levels of Complex I were strongly reduced. However, the remaining holoenzyme appeared to be fully assembled. Thus, the molecular function of FOXRED1 remained unclear. Interestingly, FOXRED1 is not present in all organisms that contain Complex I. In initial *in silico* analyses we will thus search for differences in Complex I composition between species containing FOXRED1 and species where the protein is absent. Furthermore, we will apply the following three experimental strategies: (1) Analyze the effect of FOXRED1 knockdown or overexpression (also of the disease-linked point mutant $FOXRED1^{R352W}$) using the above described assays, (2) Search for interaction partners of FOXRED1 (native immunoprecipitations) and investigate changes in the mitochondrial proteome upon FOXRED1 knockdown by quantitative proteomics (cooperation with Jörn Dengjel, Freiburg), (3) Employ purified FOXRED1 (wild type, mutants) to setup activity assays using model substrates. These assays could later be adapted to different substrates in case we should succeed in identifying these using our *in vivo* approaches.