

Daniela Vitali – Final report for Knoop Travel Award

Generally, each protein is specifically targeted to a single organelle; however, some proteins are located in different compartments where they fulfil specific functions. For example, several dually localized proteins are shared in yeast cells between mitochondria and peroxisomes. Among such proteins are the AAA ATPase Msp1, the fission protein Fis1, and the GTPase Gem1. Interestingly, it has been recently shown that the targeting of Fis1 and Gem1 to mitochondria is mediated by the cytosolic protein Pex19, known to direct transmembrane proteins to peroxisomes.

However, up to now it remains unclear how this dual localization is achieved. It is possible that a single machinery directs a protein to different compartments or that different pathways recognize the same targeting information and deliver the protein to the respective organelle.

To deepen our knowledge concerning the regulation of the dual localization of proteins to mitochondria or peroxisomes, it is essential to pinpoint factors that mediate this process. To this aim, I genomically tagged the N-terminus of Fis1 or Gem1 with a mCherry fluorophore and combined them with a C-terminally GFP tagged Om45 or Pex3, as mitochondrial or peroxisomal markers, respectively. During my stay in Prof. Schuldiner's laboratory (Weizmann Institute of Science, Israel), via an automated mating approach these four markers combinations were introduced into a yeast whole genome library of mutants, including deletions of non-essential genes and hypomorphic alleles of essential ones. Subsequently, the high throughput set up in the Schuldiner's lab allowed me to image the genomic library containing mCherry-Fis1 combined with Pex3-GFP marker and to analyse the subcellular localization of Fis1 in each deletion strain. This analysis enabled to identify some interesting factors altering the dual localization of Fis1, which will be confirmed by the examination of the other three libraries.

The identification of novel factors involved in the dual localization of proteins in mitochondria and peroxisomes will provide a better understanding of the molecular mechanisms regulating the targeting of proteins to specific organelles and the cross talk between the different targeting machineries. Moreover, this experience allowed me to increase my scientific background and gain knowledge on automated mating system and robotic microscopy set up in the laboratory of a world leader expert in such systematic screen as Maya Schuldiner. The research stay in her laboratory has been a great opportunity to collaborate with and get feedbacks from scientists working on a similar field in an international environment and gave me the chance to establish possible future collaborations, essential for a career in academia. Collectively, the research stay has been a unique chance for me to expand my scientific, intellectual, and cultural horizons.