Summary

Biogenesis of mitochondrial signal-anchored proteins - from early cytosolic events to their membranal integration

Dr. Layla Drwesh

Mitochondria harbour proteins with different topologies in their outer membrane (OM). In this study, I focus on one topological protein category, known as signal-anchored (SA) proteins. This family consists of proteins that span the membrane once via a single helical TMS located at their N-terminus, exposing a large C-terminal domain towards the cytosol. Like all mitochondrial OM3 proteins, SA proteins are encoded by nuclear DNA, translated by cytosolic ribosomes, and then are targeted to the organelle. Assuring proper and efficient targeting of such proteins is crucial for maintenance of mitochondrial biological function. Despite their importance, our understanding of the import routes that SA proteins follow including the early cytosolic events is scarce.

In this study, I aimed to unravel the biogenesis steps of SA proteins following their synthesis in the cytosol until their recognition at the mitochondrial surface. To this end, I have applied a wide set of *in vivo*, *in organello*, and *in vitro* assays using various mitochondrial SA substrates as model proteins.

I found that the MIM complex is required for the membrane insertion of the SA quality control protein Msp1, while other proteins from this category appear to follow different routes. These findings suggest that proteins from the same category may not necessarily follow the same pathway, but rather rely on different import factors to varying degrees.

To shed light on the early cytosolic events that are essential for maintaining the newly synthesized SA proteins in an import competent conformation, I analysed the involvement of some cytosolic chaperones in their early biogenesis stages. I found that chaperones from distinctive families interact with newly synthesized SA proteins through the hydrophobic segments of the latter. I further could show that such interactions are not only crucial for keeping SA proteins stable in the cytosol, but also for their optimal targeting and import into the organelle.

Next, I investigated the implication of the interplay between cytosolic chaperones and mitochondrial receptors on the biogenesis of SA proteins. My findings suggest a role of the TOM complex receptors in collaboration with the Hsp40 and Hsp70 chaperones in mediating the recognition and the insertion of SA proteins.

Overall, my findings provide new insights into the early cytosolic events in the biogenesis of SA proteins following their synthesis until their recognition at the mitochondrial surface.