

Every living organism needs energy to sustain its life and to reproduce. In humans, such energy is sourced from mitochondria, which form small, indispensable powerplants for almost every cell. Perturbations in these organelles result in numerous diseases, including cancer, neurodegeneration and aberrant inflammatory response. Despite the important role, mitochondria have a comparatively small genome to encode instructions for functioning and cannot operate entirely on their own. In fact, many proteins required for optimal mitochondrial performance are made elsewhere in the cell and then imported into the organelle. One important protein in human mitochondria of extra-organelle origin is polynucleotide phosphorylase (hPNPase), a highly conserved enzyme catalysing RNA degradation. hPNPase resides mostly in the mitochondrial intermembrane space (IMS), a compartment situated between two membranes surrounding the mitochondrial matrix, but what exactly it does there is mysterious. Some hPNPase is also located in the mitochondrial matrix, where the enzyme acts in a degradative mode to destroy superfluous RNA. This degradative activity of PNPase is supported by another protein, Suv3, with which the enzyme forms a stable complex.

PNPase can also be found in bacteria, where it can degrade RNA, but was recently discovered to form a complex with a partner protein and certain RNAs that results in an activity switch from a destructive mode into and a protective one. As the human and bacterial enzymes are highly similar in sequence and structure, it is possible that hPNPase could have a dual mode of action as well, and this second activity could play a biological role in the human mitochondrial IMS. The proposed protective mode would switch on upon encountering a special RNA substrate that the enzyme would not be able to destroy, or a protein partner that could re-programme hPNPase activity.

The research I want to carry out aims at understanding the role of hPNPase in the intermembrane space of the mitochondria. My working hypothesis is that hPNPase displays two modes of action on its RNA substrates, degradative and protective, and that the protective one could act in the IMS. In contrast, hPNPase located in the mitochondrial matrix in complex with partner protein Suv3 would always act in a degradative mode. Therefore, by physical separation of hPNPase in two different compartments, both its activities could operate in the mitochondria to support organelle function.

I would like to conduct biochemical and molecular biology experiments that will determine if hPNPase can function as an RNA regulatory factor as well as an RNA degrading enzyme in the mitochondria. I also plan to determine the precise three-dimensional structure of hPNPase, alone and bound to the substrate RNA, using cryo-electron microscopy, a rapidly evolving technique allowing scientists all over the world to gain insight into different complicated macromolecular assemblies. Moreover, I will design molecular tags that would not interfere with hPNPase activity, and which allow on further characterisation of the enzyme in human cells. My planned research will help us understand the role of human PNPase in the mitochondrial intermembrane space, and with such knowledge we could be one step closer to recognise causes of some mitochondrial disorders.