Final summary report

Mutations in mitochondrial DNA (mtDNA) are known to cause a large number of genetic diseases and have also been linked to both normal ageing and neurodegeneration. In spite of this, our knowledge of the mechanisms of mtDNA deletions and repair is still very limited. The overall aim of this project was to investigate the molecular mechanism of mtDNA repair and its functional role in human disease.

During this Marie curie IOF project, Sjoerd Wanrooij has studied what is the mtDNA replisome response upon encounter of oxidative DNA damage. The data clearly demonstrate the replication system in the mitochondrial is ill equipped in dealing with these types of mtDNA damage. During the progress of this project others reported on the existence of an additional DNA polymerase located in the mammalian mitochondria (PrimPol). In agreement with this study we have confirmed PrimPols mitochondrial localization by microscopy. Furthermore we have additional data that shows a physical and functional interaction with the mtDNA replicative helicase TWINKLE. Our data demonstrates that PrimPol is able to assist the mtDNA replisome in efficiently by-passing certain types of oxidative DNA damage (eg. 8-oxoguanin) (figure 1). However, even in the presence of PrimPol other types of oxidative DNA damage (e.g. abasic sites) remains a total block for the progression of the mitochondrial replication machinery (Figure1).

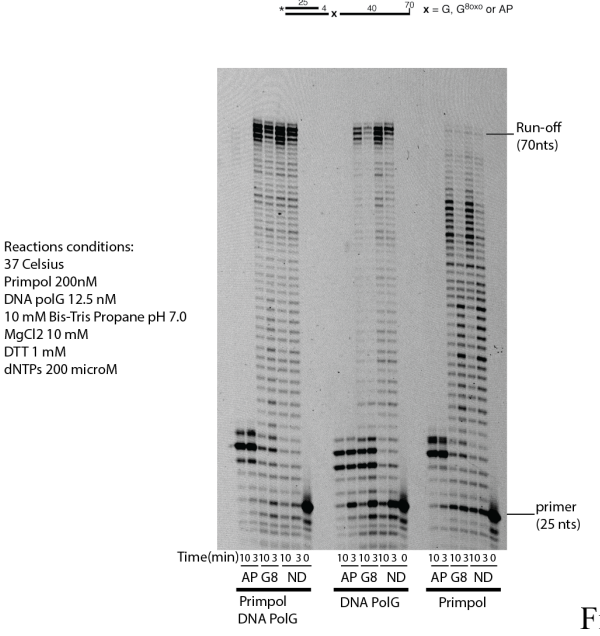

Figure 1. The mitochondrial DNA polymerase gamma has difficulty bypassing oxidative DNA damage as 8-oxoguanine (G8) and abasic sites (AP). Addition of Primpol can assist DNA polymerase gamma to efficiently bypass 8-oxoguanine. An abasic sites however remains also in the presence of Primpol a total block for the mitochondrial DNA replication machinery. PrimPol DNA synthesis on 8-oxoguanine (G8) is nearly as efficient as replication on non-damaged (N.D.) DNA template.

These results show that oxidative DNA damage can lead to mitochondrial DNA replication stalling. This stalling is the first step of mtDNA deletion formation. This is in particular of interest for mechanisms behind neurodegenerative disease. The

affected tissue of e.g. Parkinson and Alzheimer disease have an increased levels of mitochondrial reactive oxygen species. This increase is likely to result in accumulation of mtDNA damage. We now show that this increase of mtDNA damage can lead via a mechanism of replication stalling to the formation of mtDNA deletions. mtDNA deletions found in neurodegenerative disease have great influence on the disease progression.

Another objective of this Marie Curie IOF project was to identify novel mtDNA repair factors. Sjoerd Wanrooij has been able to setup a method called Isolate Proteins On Nascent MtDNA-method (iPONM). This method is a mitochondrial DNA related adaption from the IPOND method that was published by by the Cortex lab (Sirbu et al 2012 Nat. protocol). Using this method we specifically pull down proteins that associate with actively replicating mtDNA (figure 2). In this way we have identified candidate proteins that are putative mtDNA replication protein. We are now in the progress of validating this data and address the precise function of these proteins in mtDNA metabolism. We are now ready to use this optimized method to investigate what DNA repair protein are requited to the mtDNA upon induction of mitochondrial DNA damage.

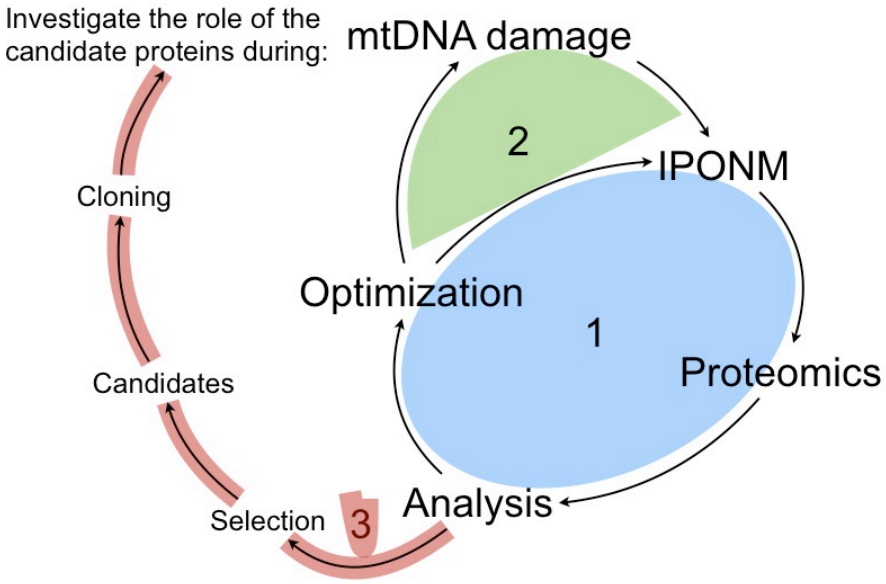

fig2. Isolate Proteins On Nascent MtDNA. After several optimization steps and proteomic analysis (1 and blue) we were able to identify several putative novel mtDNA replication proteins. We are in the process to validate these results with the candidate proteins individual and characterize the precise role of these protein in mtDNA metabolism (3 and pink). The next step is to implement this optimized protocol to investigate which protein will be requited after induction of mitochondrial DNA damage (2 and green).