Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 6B.10

OVEREXPRESSION OF DNA POLYMERASE ZETA REDUCES THE MITOCHONDRIAL MUTABILITY CAUSED BY PATHOLOGICAL MUTATIONS IN DNA POLYMERASE GAMMA IN YEAST

BARUFFINI E., SERAFINI F., FERRERO I., LODI T.

Department of Genetics, Biology of Microorgasnisms, Anthropology, Evolution, University of Parma, Viale Usberti 11/A, 43124 Parma (Italy)

DNA polymerase zeta, Rev1, DNA polymerase gamma pathological mutation, rescue of mtDNA mutability, Rev3 mutagenesis

DNA polymerase zeta (Pol zeta), which is composed by Rev3 and Rev7 subunits, and Rev1 are polymerases involved in translesion synthesis (TLS). The main role of Pol zeta during TLS is the extension from terminally (mismatched) primers. Rev1 encodes for a deoxycytidyl transferase that preferentially incorporates C opposite to an abasic site. Interaction of Rev1 with Pol zeta trough a Rev3-Rev1 binding stimulates the activity of Pol zeta. Besides their role in the nucleus, yeast Rev3, Rev7 and Rev1 localize also in mitochondria: deletion of any of the genes encoding these enzymes increases the point mutation of mitochondrial DNA (mtDNA) measured as the frequency of mtDNA mutants resistant to erythromycin (Ery^R).

It has been previously speculated that, in absence of the TLS by Rev1 and/or Pol zeta, the mitochondrial DNA polymerase gamma (Pol gamma), the mitochondrial replicase, could introduce mutations by replicating the mtDNA lesions. As a consequence, Pol zeta and Rev1, which are responsible in the nucleus for the error-prone bypass of DNA lesions, in the mitochondria could be responsible for an error-free bypass, or maybe less error-prone, compared to Pol gamma. Pol gamma is a protein conserved in fungi and animals. To date, more than 150 pathological mutations in POLG have been identified in severe mitochondrial disorders. In yeast the DNA polymerase gamma is encoded by *MIP1* gene. Thanks to the similarity between human Polg and Mip1 (approximately 43%), yeast was used to validate the role of human putative pathological mutations, to understand the biochemical consequences associated to these mutations and in particular to find mechanisms able to rescue the harmful effects of Mip1 mutations, such as the treatment with antioxidant molecules.

In this perspective, we wondered whether overexpression of Pol zeta and Rev1 could rescue the detrimental effects on mtDNA point and extended mutability caused by mutations mapping in different domains of MIP1. We show that overexpression of Rev3 reduces, in a Rev7-dependent and Rev1-independent manner, the mtDNA extended mutability caused by a subclass of pathological mutations in Mip1, the yeast mitochondrial DNA polymerase orthologous to human Polg, whose detrimental effects on mtDNA stability are not rescued by treatment with antioxidants. This beneficial effect observed is synergistic with the effect achieved by increasing the dNTPs pools, which are the substrate of both Pol zeta and Pol gamma. On the contrary, the overexpression of Rev3 does not rescue the mitochondrial extended mutability caused by Mip1 mutations sensitive to the treatment with antioxidants. Furthermore, overexpression of both Pol zeta and Rev1 reduces mtDNA point mutability in mtDNA mutator *mip1* strains. Since overexpression of Rev3 is detrimental for nuclear DNA mutability, in order to obtain the beneficial effect on mtDNA without

the negative effect on nuclear DNA, we constructed and overexpressed a mutant isoform of Rev3 unable to migrate into the nucleus: it reduced mtDNA mutability without increasing nuclear mutability.