Spontaneous mutations produce the genetic variation that drives evolution in all organisms, but at the same time, they affect fitness and are harmful for the organism.
The mechanisms by which organisms efficiently and faithfully replicate their DNA are a subject of major scientific interest. One area where understanding the mechanisms of fidelity of DNA replication might be particularly helpful is the analysis of the sources of genetic instability. This last issue is essential in understanding the cancer etiology.
Recently, it has been postulated that the lifetime risk of many types of cancer is strongly correlated with the number of divisions (rounds of replications) of the normal cells maintaining that tissue's homeostasis. Obtained results suggest that majority (70%) of cancers are due to "bad luck", that is, random, spontaneous mutations arising during DNA replication in normal cells.
In most organisms, the accuracy of DNA replication is determined by three highly conserved processes: correct base selection by DNA polymerases, removal of the wrong nucleotides proofreading activity of DNA polymerases, and postreplication correction of polymerase errors by the DNA mismatch repair system. Thus, replicative DNA are central to replication fidelity. However, recently the contributions of other factors that may influence the final fidelity of DNA replication and the level of spontaneous mutagenesis are specific issue of interest. These factors include: noncatalytic subunits of DNA polymerase holoenzymes, mechanisms underlying the differential replication fidelity of both DNA strands and finally the involvement of other accessory DNA polymerases that have been discovered and characterized in recent years. The yeast Saccharomyces cerevisiae has eight, and human cells possess at least sixteen DNA polymerases.
In all organisms DNA replication is carried out by a multiprotein complex referred to as the replisome. The primary scientific goal of this project is understanding of the role of the noncatalytic subunits of the replisome in targeting of different DNA polymerases into the replication fork and how changes affecting this process influence DNA replication fidelity and in consequence the level of spontaneous mutagenesis.
In eukaryotic cells DNA replication relies on coordinated DNA synthesis by three replicative DNA polymerases: Pol alpha, Pol delta and Pol epsilon. As the two strands of DNA are replicated in different fashion, the contribution of the activities of the two major replicative DNA polymerases (Pol delta and Pol epsilon) to leading and lagging strand replication is a central question in the field of eukaryotic DNA replication. To date there is no definite answer to this question. This knowledge is essential in our understanding of the mechanism responsible for faithful genomic replication and how mutant forms of DNA polymerases lead to the mutator (increase number of mutations) phenotype of the cells. Recent in vitro study has shown that interaction of Pol epsilon with CMG helicase complex (helicases are motor proteins that move directionally along a DNA or RNA, separating two annealed nucleic acid strands) might be responsible for loading of Pol epsilon on the leading strand. To verify this hypothesis in vivo we are going to use yeast strains carrying mutant forms of Pol epsilon and GINS complex (part of CMG helicase). We want to determine how altered interactions between Pol epsilon and GINS complex affect specific loading of DNA polymerases and to what extent this influences the level of spontaneous mutagenesis. Our preliminary data suggest that changes in the division of labor at the replication fork strongly elevate the level of spontaneous mutagenesis. Obtained results are essential in understanding the role of noncatalytic elements of the replisome as genetic stability enhancers.