

DISSERTATION SUMMARIES

***In vitro* analysis of the regulation of the human DNA damage tolerance pathway**

Dávid Balogh

Laboratory of Mutagenesis and Carcinogenesis Research, Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

The DNA in our cells is continuously damaged by different agents, such as UV irradiation, reactive oxygen species, metabolites and chemicals. These agents are changing the structure of the DNA molecule, leading to mutations during their replication. To avoid these mutations many DNA repair mechanisms have evolved. These mechanisms are able to set back the original structure of the DNA double helix but some damages get to the S phase of the cell cycle where they can cause the stalling of the replication fork, chromosomal breaks and cell death. To avoid these possibilities the DNA damage bypass pathway has evolved which can protect the stalled replication fork by different ways.

The main step of the pathway is the monoubiquitylation of the PCNA protein, which is the processivity factor of the polymerases by Rad6/Rad18 complex at the lysine 164 position. After this modification the replicative polymerase can be changed by an alternative polymerase, which is able to synthesize through the lesion. In an other error free mechanism the monoubiquitylated PCNA becomes polyubiquitylated by the Mms2/Ubc13/HLTF complex through lysine 63 residues, which can facilitate HLTF dependent replication fork reversal. On this newly emergent so-called chicken foot structure the stalled replication can be rescued using the newly synthesized sister strand as a template. The third possibility is an alternative template switching mechanism.

Our study is focusing on the better understanding of the function and regulation of the DNA damage bypass pathway. A stalled replication fork is surrounded by various DNA-binding proteins which can inhibit the access of damage bypass players, and it is unknown how these proteins become displaced. We found that HLTF has an ATP hydrolysis-dependent protein remodeling activity, by which it can remove proteins bound to the replication fork. Our ultimate goal is to shed light on the whole molecular mechanism of the damage bypass.

Supervisor: Lajos Haracska
E-mail: balogh-dave@gmail.com

The consequence of PrP^C or Shadoo overexpression on the cytotoxic effect of a Δ CR-PrP mutant phenotype

Petra G. Bencsura

Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Transmissible spongiform encephalopathies (TSE) are lethal neurodegenerative disorders with characteristic spongiform degenerations of the brain and variable degree of amyloid plaque formation. TSE is caused by the conformational transition of the cellular prion protein (PrP^C) to an abnormal isoform, commonly referred as PrP^{Sc}. PrP^C is a glycoprotein on the cell surface, anchored to the outer leaflet of the plasma membrane by a glycosylphosphatidyl-inositol (GPI) moiety. PrP^C expressed ubiquitously in the body reaching highest levels in the central nervous system and the heart. In TSE neurotoxicity is coupled to transmissibility.

However, with deletion mutant PrPs that are expressed in PrP knockout mice, the toxicity and the transmissibility can be separated: (i) Upon infection of transgenic mice expressing GPI-anchorless PrP, PrP^{Sc} is formed but the mice did not show any sign of neurodegeneration. Thus, the formation of GPI-anchorless PrP^{Sc} is not neurotoxic, although anchorless PrP^{Sc} can cause neurodegeneration in wild type mice when transmitted into their brains. (ii) In case of internal deletion PrP mutants, the expression of the mutant proteins is neurotoxic, the mice develop a neurodegenerative phenotype that is reminiscent of that seen in TSE, but this prion disease is not transmissible and the deletion mutant proteins do not convert to an abnormal conformation. Understanding the mechanism of the neurotoxic effect of the internal deletion mutant prion proteins might help to understand the mechanism of the neurotoxicity of the conformational conversion of PrP^{Sc}. Additional advantage of this model system is that no infectious agent involved, that simplify several steps in the research project.

The deletion mutant of the prion protein missing the segment, called Central Region, referred as Δ CR mutant, causes a neonatal lethal phenotype when expressed in PrP knockout mice, provides an alternative approach in the understanding of the physiological function of PrP^C and how PrP^C can be degraded to produce neurotoxic effects. Expressing Δ CR-PrP, in mammalian neural cells has been shown to cause hypersensitivity to the toxic effects of antibiotics using for stable cell line selection. This hypersensitive phenotype can be rescued by co-expression of wild type PrP^C.

Our aim is to establish a model system for studying the toxic effect of Δ CR-PrP in mouse neuronal N2a and human neuronal SH-SY5Y cells, for examining whether Shadoo (Sho, a member of the Prion protein family) like wild type PrP can also rescue this phenotype and for discerning the underlying mechanism.

We established stable cell lines, which expressed the Δ CR-PrP mutant or wt PrP^C with the reporter gene GFP. Subsequently, in order to achieve a high overexpression of wt PrP^C or Sho in the Δ CR-PrP expressing cells lentiviral transduction is used. For assessing the drug hypersensitivity caused by the expression of Δ CR-PrP, the cell viability with or without Zeocin treatment is measured using an MTT assay.

Supervisor: Dr. Ervin Welker
E-mail: bencsura@brc.hu

A comprehensive view of the determinants of molecular evolution in yeast

Gábor Boross

Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Why do genes evolve at different rates? It is a well-known phenomenon that amino acid sites of protein sequences undergo substitutions during the course of evolution, and the rate of this change varies widely across genes. In the past years the major determinants underlying protein sequence evolution have been largely uncovered. However, molecular evolution is not restricted to amino acid substitutions, but rather encompasses various aspects of molecular changes from the deletion and duplication of whole genes to change in expression levels and subcellular localizations. Importantly, while the rate of sequence and expression divergence has been thoroughly studied, gene duplicability and the propensity for gene loss remain poorly understood, and it remains completely unexplored what determines the propensity for change in subcellular localization.

Here we aim to systematically explore and compare the genomic and functional genomic properties determining i) sequence divergence, ii) gene expression divergence, iii) propensity for gene loss, iv) gene duplicability and v) propensity for change in subcellular localization of proteins.

We compiled a dataset of various evolutionary variables (*i.e.* evolutionary rates of the above-listed molecular traits) using available information on sequence, expression, gene annotation and protein localization from *Saccharomyces cerevisiae* and its homologous genes in related species ranging from *S. paradoxus* to *S. pombe*. We also compiled high-coverage functional genomic data on various genomic and functional properties of genes/proteins in *S. cerevisiae* (e.g. information on protein abundance, protein network connectivity, fitness contribution, genetic interaction connectivity, etc.). In addition to classical statistic tools, we employed a data mining regression tool (random forest) to predict evolutionary rates based on these gene properties. This enables us to compare the predictability and the main determinants of different aspects of molecular evolution.

First, we asked whether the different molecular traits of a gene evolve in a correlated fashion. We found that the different evolutionary rates show only very weak correlations with each other, suggesting that different gene properties diverge rather independently throughout evolution. Next, we examined the predictability of molecular evolution. Corroborating earlier reports, we found that sequence divergence is well-predictable, with 54% of variation in divergence rate explained. The rate of expression divergence and gene loss can also be predicted using genomic features (28% and 27%). Duplicability, however, showed little predictability. Furthermore, in contrast to other evolutionary traits, the rate of duplication is only marginally conserved when calculated on different branches of the phylogenetic tree. Taken together, these findings indicate that gene duplication is not driven by strong universal evolutionary forces. We found that the rate of evolutionary divergence in protein localization is also predictable and revealed novel factors determining the conservation of protein subcellular localization. For example, we found that highly expressed genes show especially strong conservation in localization.

In our study, we have systematically examined the driving forces behind evolutionary change of different gene-level molecular traits using data-mining methods and recent functional genomic datasets. While some evolutionary variables are highly predictable, we report that the diversity of duplicability across genes is lineage-specific and no strong universal determinant of duplicability exists. We also discovered a number of novel determinants of protein localization conservation.

supervisor: Balázs Papp
E-mail: borossg@brc.hu

Analysis of blood cell lineages in *Drosophila melanogaster*

Gábor Csordás

Immunology Unit - Laboratory of Immunology, Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Insects are armed with a powerful innate immune response, which provides an effective barrier against invaders and tumors. The phylogenetically conserved immune functions, such as the phagocytosis of microbes and the encapsulation of large foreign particles are carried