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DISSERTATION SUMMARIES

DNA Replication across the protein-DNA adduct

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In cells, DNA is tightly associated with a variety of proteins that serve both to maintain the structural organization of the genetic material and to coordinate cellular processes including replication, repair, recombination, and transcription. Many endogenous compounds (e.g., metabolites of lipid peroxidation) as well as environmental agents are reactive with both DNA and proteins and thus can produce covalent linkage between these two types of macromolecules.

DNA-protein cross links (DPCs) arise in biological systems as a result of exposure to a variety of chemical and physical agents, many of which are known or suspected carcinogens. These DPCs formed within the cells are usually removed/ cleaved by different cellular mechanisms. The unresolved DPCs can hinder normal functioning of a cell by blocking regular cellular mechanism like DNA replication, transcription and others.

Despite the recognition of the biological significance of DPCs, there are very limited data concerning the repair of these lesions. One possible hypothesis is that the covalent or irreversible bondage of a protein to DNA somehow modifies the whole structure of DNA double helix and hence allowing cell to recognize these DPCs as unnatural nucleotide base pair. . The mechanism how a cell recognizes these DPCs and how these unnatural structures are resolved still remain to be unclear.

Analyses of data generated in prokaryotes revealed the existence of mechanisms of active DPC removal and suggested that more than one repair pathway can be involved in the repair of these lesions. There are couple of possible hypotheses , one being the protein part of the DPCs is to be degraded/ cleaved specifically by a protease, and other Nucleotide excision repair (NER), the repair mechanism in which a damaged base is cleaved and replaces by a regular nucleotide bases. However all the hypotheses lack a proper experimental system. It has been previously reported that DNA replication machinery fails to replicate the DNA in the presence of DPCs revealing the fact of stalling the DNA replication fork at the Site of DPCs. However the exact mechanisms how an ongoing replication fork can bypass these DPCs is largely unknown due to lack of a proper in-vivo or in-vitro experimental system.

In the present study we are developing an in-vitro system to monitor stalling or bypass of DNA replication machinery at the site of DPCs. To accomplish the above task a suicidal DNA substrate is designed to trap a protein irreversibly. DNA binding or DNA modifying proteins can be used to crosslink to the DNA of known sequence. This cross-linked DNA-protein substrate is further purified and can be used as a template for the DNA replication. By using different DNA polymerase including some of the specialized TLS (translesion synthesis) polymerase which specifically replicates damaged DNA; it is possible to check bypass of these DPCs. In future these experiments will also reveal whether a specific polymerase is involved to resolve this kind of naturally occurring cross links.

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Data to the analysis of paleopathology of the Medieval Age in the region between the Danube and Tisza rivers (preliminary report)

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Human paleopathology can be defined as the study of diseases in ancient populations by the examination of human remains (dry skeletons and mummies). However, the anthropological study of diseases in antiquity is very complex and challenging. The interplay of many variables – host resistance, pathogen virulence, cultural practices, ecological settings, malnutrition, crowding – needs to be considered.

The aim is of the investigation is to perform a complete comparative analysis of populations dated to the 11th-17th centuries in the region between the Danube and Tisza rivers based on the presentation and evaluation of the paleopathological alterations.

The following series were included in this study: Nyárlőrinc-Hangár utca, Kalocsa-Szentháromság tér, Kalocsa-Belvárosi Iskola,

Bácsalmás-Mosztonga, Dunapataj-Szent Tamás domb. The samples contain the remains of 756 individuals (163 males, 54 females, 207 undetermined, 332 subadults). This skeletal material is collected at the Department of Anthropology, University of Szeged.

The specimens have been analysed for the determination of the age at death and sex and scored for the measurements. Concerning the pathological conditions, the macro-morphological examination was completed - in some cases - with radiological analyses. In one case the molecular analysis was carried out to estimate the DNA of *Mycobacterium tuberculosis.* (This investigation was made at the München University - Institute of Pathology.)

The following disorders have been identified: traumatic lesions, specific and non-specific infections, haematological anomalies, joint diseases, bone-tumor and tumor-like anomalies, developmental disorders, and enthesopathies.

It is the most important to highlight the cases of skeletal tuberculosis (one case) and –syphilitic lesions (two cases) (Nyárlőrinc-Hangár utca; Pálfi et al. 1997; Balázs et al. 2005), for these diseases were among the most important selective factors in human populations in antiquity. In the sample Dunapataj-Szent Tamás domb, the frequency of the developmental anomalies is very significant by the reason of endogamy (Balázs and Marcsik 2007b).

In the Nyárlőrinc-Hangár series (11th-17th centuries; V. Székely 1987), there was excavated a partly mummified foetus which was buried in a crock at the edge of this cemetery and dated to 19th century on the basis of a copper coin which was put into the crock (Balázs and Bölkei 2007a).

This presentation is only a preliminary result.

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Functional characterization of the plant SET protein: from phosphatase inhibition to heat stress tolerance

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Even small environmental changes can induce expression or repression of hundreds of genes in plants, contributing to their endless adaptation to the changing environment. Regulation of such a synchronized genomic event has to employ chromatin remodelling – a process that involves post-translational modifications of histones. One of the putative proteins involved in the regulation of histone modification patterns is SET. SET, belonging to the NAP/SET family of potential histone chaperones, is a multifunctional protein involved in very diverse cellular processes in mammals.

It was previously shown that human SET inhibits protein phosphatase 2A (PP2A) (Li et al. 1996), a major serine/threonine phosphatase both in plants and animals. It was also demonstrated that SET is associated with transcriptionally active loci in response to heat shock in *Drosophila melanogaster*, and these regions encoding heat shock proteins are marked with phosphorylation of histone H3 at serine 10 (Nowak et al. 2003).

Although the members of the NAP/SET family are well characterized proteins in animals (reviewed in Park and Luger 2006), we have little information on the plant NAP1 (nucleosome assembly protein1)-related proteins. The aim of our studies was hence the characterization of the *Arabidopsis* SET protein.

Our results revealed that the recombinant *Arabidopsis thaliana* SET protein exhibited inhibitory effect on the activity of purified preparations of rabbit PP2A and PP1 (protein phosphatase 1) catalytic subunits against a phospho-histone substrate. In addition, purified SET inhibited the dephosphorylation of histone H3 at serine 10 position by immunoprecipitated *Arabidopsis* PP2A and interacted *in vitro* with purified calf histone H3.

Phosphorylation of serine 10 on histone H3 is coupled with two opposite chromatin states: it is associated with mitotic chromosome condensation, while it occurs also during interphase in correlation with transcriptionally active loci (Johansen and Johansen 2006). Since our results suggest that SET may have a role in the maintaining of this kind of histone modification in plants, we propose a role for SET in transcriptional regulation. The verification of the involvement of the *Arabidopsis* SET in gene expression control, however, needs further investigations.

We also demonstrated that the subcellular localization of SET was influenced by a heat stress treatment at 45°C. In response to heat, SET accumulated in the nucleus, while under standard conditions it is located predominantly in the cytosol. Interestingly, other types of stresses including heat stress at lower temperature (37°C), salt stress, heavy metal stress or genotoxic stress did not cause the nuclear accumulation of SET, suggesting a specific role for SET in certain plant stress responses.