DNA repair and antigenic variation in *Trypanosoma brucei*

Ulbert, S.

Citation for published version (APA):
Ulbert, S. (2003). DNA repair and antigenic variation in *Trypanosoma brucei*
Summary

DNA modification is a common phenomenon in nature, it occurs in prokaryotes and eukaryotes. Modified DNA bases have various functions, ranging from protection mechanisms against foreign DNA to complex roles in the control of gene expression. In Trypanosoma brucei, a unicellular eukaryotic parasite that shuttles between mammals and insects, 1% of the thymine residues in nuclear DNA is replaced by the modified base β-D-glucosyl-hydroxymethyluracil (J), mostly in repetitive telomeric sequences. J is only detectable in the bloodstream form of the parasite, and the function of this DNA modification is not known yet. In addition to J, T. brucei DNA contains small amounts of 5-hydroxymethyluracil (5-HmU). Previous results suggested a model in which J is synthesized in two steps at the DNA level, with 5-HmU as an intermediate. However, no J-synthesizing enzymes have been identified to date. To gain further insight into J-biosynthesis and the role of 5-HmU, we established a system to specifically remove 5-HmU from DNA by integrating the gene for the human DNA glycosylase hSMUG1 into T. brucei (Chapter 2). hSMUG1 functions in the base excision repair (BER) system and excises 5-HmU, generating an abasic site in DNA, which is further processed by other BER factors. The expression of the gene in T. brucei led to a decrease in J-content of the cells. Furthermore, hSMUG1 caused an accumulation of abasic sites and double strand breaks in DNA due to excessive removal of 5-HmU, leading to an arrest in cell cycle and eventually death of the trypanosomes. This DNA damage was specific to J-modified sequences indicating that 5-HmU colocalizes with J. Expression of hSMUG1 in insect form T. brucei had no effect on the cells. This showed that, similar to J, 5-HmU is only present in the bloodstream form of the parasite (Chapter 3). These results are consistent with the idea that 5-HmU is a precursor of J and confirm the two-step model for J-biosynthesis. We also looked directly for J-synthesizing enzymes by performing in vitro J-biosynthesis assays with trypanosome extracts (Chapter 4). These experiments yielded inconclusive results, as the activity identified could not be confirmed by using another, independent approach.

The data obtained with hSMUG1 indicated that a BER activity against 5-HmU would be harmful to trypanosomes. We addressed this question further by investigating whether the presence of J in T. brucei has led to adaptations in the BER system of the parasite (Chapter 5). DNA glycosylases, the major components of BER, are extremely conserved in evolution, and it was not known whether some of these enzymes would excise J. Hence, T. brucei might lack some DNA glycosylases in order to tolerate J in its DNA. By performing biochemical BER assays, we tested
Summary

the ability of several different DNA glycosylases from various origins to excise J or 5-HmU from DNA. No excision of J was found, but 5-HmU was excised by AlkA and Mug from *Escherichia coli* and by human SMUG1 and TDG. In a combination of database searches and biochemical assays, we identified several DNA glycosylases in *T. brucei*, but we detected no excision activity in trypanosome extracts towards 5-HmU or ethenocytosine, a product of oxidative DNA damage and a substrate for Mug, TDG and SMUG1. These results indicate that trypanosomes have a BER system similar to that of other organisms, but might be unable to excise certain forms of oxidatively damaged bases. The presence of J in DNA does not require a specific modification of the BER system, as this base is not recognized by any known DNA glycosylase. These results suggest that J evolved without disturbing BER and are analogous to data obtained with other DNA modifications such as 5-methylcytosine, which is also not removed by DNA repair.

In an independent line of experiments, we investigated the phenomenon of antigenic variation in *T. brucei*. In order to escape total destruction by the mammalian immune system, the parasite repeatedly changes its surface coat, which consists of a dense coat of a single protein, the variant surface glycoprotein (VSG). There are about a thousand different VSG genes and in order to be transcribed they have to be located in one out of twenty highly homologous VSG gene expression sites. Expression sites are subject to allelic exclusion, resulting in only one expression site being active at a given time. One mechanism to change the expressed VSG gene is to inactivate one expression site and to activate another one (*in situ* switch). We generated trypanosomes with three expression sites tagged with three different drug resistance genes (Chapter 6). By performing drug selection experiments with this cell line, we investigated a previously identified, putative intermediate state of the *in situ* switch. The results showed that during the *in situ* switch, two expression sites enter a short-lived state termed “pre-active”, which leads to a transient activation of both expression sites, whereas the others remain transcriptionally silent. All three expression sites analyzed entered the pre-active state at similar frequencies suggesting that it is a general feature of the *in situ* VSG switch. We also used the cell line containing three marked expression sites to investigate the transcriptional silencing of inactive VSG expression sites and could show that expression site regulation is a dynamic process and that the level of transcription at inactive sites can be altered, leading to partially activated expression sites. In addition, we tried to localize the active expression site in nuclei of living trypanosomes by inserting a system for *in vivo* GFP-labelling of DNA into *T. brucei* (Chapter 7). This approach was complicated by the fact that the repetitive sequences necessary for the *in vivo* labelling were unstable in the trypanosomes and were rapidly lost from the DNA.