6 SUMMARY

Apoptosis exhibits important functions in the development, differentiation and homeostasis of tissues and organs in all multicellular organisms. This cellular suicide program eliminates excessive and infected cells as well as cell with irreparable DNA damages. In the mammalian immune system, apoptosis is an important mechanism that prevents the development of non-functional and autoreactive lymphocytes. The programmed cell death of autoreactive B lymphocytes, mediated by self-antigens *in vivo*, can be reproduced in cell culture by crosslinking the B cell receptors of the human Burkitt's lymphoma cell line BL60-2 with anti-IgM antibodies which leads to apoptosis in over 70% of the cells after 24 hours.

In the present study, I detected a profound downregulation of proliferation after induction of apoptosis in BL60-2 cells and consequently analyzed the impact of apoptosis on the pre-replicative complex (pre-RC), which is essential for the initiation of DNA replication. A reduction on mRNA level for the pre-RC components Mcm2 – Mcm7 and Cdc6 early in the apoptotic process was revealed in an RNase protection assay. In addition to the reduction of gene expression, an apoptotic cleavage for two of the corresponding proteins, Mcm3 and Cdc6, was detected. The cleavage of both proteins seems to be a general phenomenon in apoptotic cells, as it was detected in three distinct cell lines and in response to diverse apoptotic stimuli.

The apoptotic cleavage of Mcm3 and Cdc6 is mediated by caspases. The involved effector caspases as well as the respective cleavage sites were determined for both proteins *in vitro*. Mutation of the identified cleavage sites in Mcm3 and Cdc6 generated proteins which were not cleaved upon the overexpression and subsequent induction of apoptosis in HeLa cells, while overexpressed wildtype Mcm3 and Cdc6 were processed like the endogenous proteins. The cloning and overexpression of the apoptotic Mcm3 and Cdc6 fragments in HeLa cells allowed the analysis of their respective intracellular localization and their capacities to form complexes with Mcm4 (Mcm3 fragments) or to bind chromatin (Cdc6 fragments) indicating their functionality in comparison to the wildtype proteins. A pro-apoptotic effect was revealed for the N-terminal Mcm3 fragment generated in apoptotic cells, indicating a possible function for Mcm3 in a positive feedback loop in apoptotic cells.

Apoptosis is an active process, which in contrast to necrosis, depends on *de novo* transcription and translation of genes needed to carry out the apoptotic degradation processes. On the other hand, anti-apoptotic proteins have to be degraded or inactivated and their *de novo* expression needs to be repressed. To investigate the differential

expression of genes in apoptotic compared to normal BL60-2 cells, I analyzed mRNA from unstimulated cells as well as cells treated with anti-IgM antibodies on Affymetrix GeneChips, cDNA membranes, and in RNase protection assays. Above all, a significant decrease on mRNA level for six genes involved in DNA repair was identified in apoptotic compared to non-apoptotic cells. Two of the corresponding proteins are also enzymatically cleaved during the process of programmed cell death, indicating that the shutdown of DNA repair is important for apoptotic cells. DNA repair in apoptotic cells would be counterproductive as the fragmentation of chromosomal DNA is an important part of the apoptotic degradation of the cell. Furthermore, DNA repair consumes ATP, which is needed for the apoptotic processes. Differential mRNA levels were also observed for transcription regulators and phosphatases as well as genes belonging to other functional groups.

Taken together, I was able to show the downregulation of important components of DNA replication and DNA repair in apoptotic cells on protein and mRNA level, in which some of the differentially expressed transcription regulators may also be involved. Thus, in advance of DNA fragmentation, caspases seem to ensure that cells destined for cell death inactivate proteins involved in maintaining genome integrity and replication, thereby forcing the cell to rapidly abort DNA synthesis in order to efficiently proceed with the irreversible degradation of its DNA. Furthermore, the downregulation of futile DNA replication and repair prevents the waste of ATP. Preserving ATP is important, as apoptosis is an energy-dependent process and an early ATP-depletion was shown to change apoptotic to necrotic cell death, a scenario that needs to be prevented as the necrotic lysis of cells causes unfavorable side-effects like inflammation. In addition, I was able to show a pro-apoptotic effect for an apoptotic Mcm3 fragment. By the induction of an amplification step, the caspase-mediated cleavage of pre-RC components fulfills an important function in the apoptotic cell, exceeding the inhibition of DNA replication. A perturbance of DNA replication due to the cleavage of pre-RC components might activate the DNA replication checkpoint, thereby inducing an apoptosis promoting signal.