APPENDIX &

SUMMARY
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SUMMARY

To cope with DNA damages induced by endogenous and exogenous agents and by spontaneous decay of nucleotides, cells employ both DNA repair and DNA damage tolerance (DDT) mechanisms. Translesion synthesis (TLS) is an important DDT mechanism in mammalian cells. Mammalian TLS is performed by at least five key proteins, *i.e.* TLS DNA polymerases Rev1, η, ι, κ and ζ. These TLS DNA polymerases play roles in bypassing unrepaired DNA adducts during and after S-phase, thereby allowing completion of genome duplication. However, since TLS is a mutagenic process, it must be tightly controlled. Thus far, the *in vivo* role of each TLS polymerase in response to DNA damages in mammalian cells and organisms has largely remained unclear. Furthermore, the relative contribution of each TLS polymerase is also poorly understood. In this thesis, I have used cell lines with single or combined deficiencies in TLS polymerases to explore the absolute and relative *in vivo* contributions of these TLS-polymerases in response to DNA damages induced by food-derived genotoxins and UV light. Furthermore, I have studied the genetic and cellular consequences of unreplicated DNA lesions, resulting from defects in TLS. Using TLS-defective mice, I have addressed the importance of TLS in preventing organismal premature aging.

Chapter 1 provides an introduction to TLS, the main players in this DDT pathway and the different mechanisms by which TLS polymerases may replicate damaged DNA.

Chapter 2 describes the *in vivo* roles of TLS polymerases in response to the food-derived genotoxins Benzo[*a*]pyrene diepoxyde and Hydroxynonenal (HNE). It was found that Rev1, Polζ and Polη play a central role in TLS when cells are exposed to either one of both agents. Consistent with *in vitro* studies, Polι is specifically involved in TLS across HNE-induced DNA damages *in vivo*. Interestingly, in addition to the known role of Polκ in TLS of BPDE-induced DNA lesions, the BRCT domain of Rev1 is crucial for bypassing BPDE-induced DNA lesions and for quenching DNA damage signaling, although this domain appears to be dispensable for TLS of HNE-induced DNA damages. The study suggests that structurally different DNA lesions are bypassed by various sets of TLS polymerases or require different functional domains of the same polymerase. Failed TLS leads to activation of DNA damage signaling, double-strand DNA breaks (DSBs) and, eventually cell death.

The *in vivo* roles of TLS-related genes in MEFs in response to UVC light are described in **Chapter 3**. UVC light induces CPDs and (6-4)PPs that may be considered as models for mildly and severely helix-distorting DNA lesions, respectively. Using similar doses of UVC light, it was found that Polι, Polκ and the BRCT domain of Rev1 are largely dispensable for the responses to UVC-induced DNA lesions, including cell survival and DNA damage response (DDR). Polη is mainly required for bypassing CPDs, while Rev1 and Polζ are essential for bypassing (6-4)PPs. Deficiencies of these TLS polymerases result in enhanced DNA damage signaling and reduced cell cycle progression. Mono-ubiquitination of PCNA (PCNA-Ub) is predominantly involved in TLS for both CPDs

and (6-4)PPs at early after exposure; however, PCNA-Ub is dispensable for bypassing (6-4)PPs late after exposure, suggesting the existence of PCNA-Ub independent TLS. The results in Chapter 3 suggest the existence of a back-up pathway that restores DNA replication in Polη-deficient MEFs after UVC irradiation. To gain further mechanistic insights, MEF lines with single, double and triple disruptions of genes encoding Pols η, ι and κ were investigated under the same experimental conditions. These experiments are described in **Chapter 4** and the results show that Polκ is a major TLS polymerase in TLS across both CPDs and (6-4)PPs in Polη-deficient MEFs. Interestingly, the triple-mutant cells displayed a more severe defect in TLS at (6-4)PPs compared to Polη-deficient MEFs with an additional defect in either Polι or Polκ. This finding suggests that multiple TLS polymerases play back-up roles in TLS at severely helix-distorting DNA lesions.

Previously, it was reported that TLS-deficient cells accumulate single-stranded DNA (ssDNA) gaps after exposure to DNA damaging agents such as UVC light. In **Chapter 5** the fate of these ssDNA gaps throughout the cell cycle was analyzed using a comprehensive experimental approach. The results unambiguously show that unreplicated (6-4)PPs within ssDNA gaps generated in both TLS-deficient and TLS-proficient replicating cells, are transmitted through mitosis to the subsequent cell cycle. In the ensuing S phase, these ssDNA gaps are converted into double-strand DNA breaks, the most cytotoxic DNA lesions when not repaired, resulting in the formation of micronuclei (MN). With regard to genotoxicity testing, this study provides a mechanistic basis for the recommendation towards the Organisation for Economic Co-operation and Development to analyze micronuclei formation during two cell cycles after exposure.

Chapter 6 describes a role of DNA replication stress at endogenous DNA lesions in premature aging. Rev1 mutant mice display age-related phenotypes earlier than wild-type mice. These aging-related phenotypes were further accelerated when Rev1-deficient mice were also deficient for Xpc, a protein involved in nucleotide excision repair. These data imply that replicative stress at unrepaired endogenous DNA damages leads to aging. The study furthermore provides experimental evidence that Rev1 acts in TLS across lipid peroxidation-induced bulky DNA lesions that mimic naturally occurring adducts. In this way, Rev1 suppresses replication stress and premature aging.

Finally, a perspective building on, and extending, the results of the studies described in this thesis is provided by **Chapter 7**.