ENGLISH SUMMARY

The genetic information required for the generation of cellular components and their organization is fixed in our genes. It is therefore important to ensure that all cells in the body function properly and that their genetic information remains intact. Various physical and chemical agents within our environment (such as UV radiation from the sun) or chemical entities (such as reactive oxygen species) generated by metabolic processes in our cells interact with the carrier-molecule of genetic information namely the DNA and cause DNA damage that can lead to mutations. The accumulation of errors in our DNA can cause a cell to divide uncontrollably and to become cancerous. Hence to avoid these adverse effects cells trigger a series of defense mechanisms upon DNA damage induction, which is collectively referred to as the DNA damage response (DDR). The DDR involves distinct DNA repair mechanisms and coordinates these processes with cell cycle progression to promote efficient and accurate removal of damaged DNA from the genome. However, while many of the key factors involved in the DDR have been identified and characterized, our current view on the interplay between the different factors and processes involved in the DDR is limited. The objective of this thesis is therefore to improve our understanding of this crucial protection program. To achieve this goal, we used a high throughput genetic screening approach called Epistatic MiniArray Profiling (EMAP). This technique can measure epistatic (positive) and synergistic (negative) interactions between pairs of genes. To assess the changes in genetic interactions induced by certain types of DNA damage, we generated EMAPs under three different DNA damaging conditions. These genetic interaction networks revealed numerous genes (and thus proteins), including well-known genes as well as several novel genes, that interact and collaborate when cells are exposed to DNA damaging agents. We have used genetic, biochemical and microscopical approaches to investigate the roles of some of these genes in the DDR.

In chapter 2, we describe the principle of EMAP, a technology that we further developed to be able to measure genetic interactions in unperturbed conditions, as well is in the presence of different DNA damaging agents. We show that our multi-conditional EMAP is a useful resource that not only reports on many well known, but also several novel factors and pathways involved in the DDR. For example, we found that camptothecin (CPT), an agent that causes double strand-breaks (DSB) during DNA replication, increase the interactions for genes involved in DSB repair. On the other hand, methyl methanesulfonate (MMS), a drug that induces base damages and the stalling of replication forks, increases interactions for post-replication repair (PRR) genes. These results validated the quality of our dataset. Importantly, we demonstrate that this type of screening is more powerful than single mutant sensitivity screens or expression profiling in highlighting associations between damaging agent and DDR pathway. Remarkably, the three distinct DNA damaging agents each induced a unique set of genetic interactions, suggesting that the lesions generated by each agent trigger a highly specific DDR. Only few genetic interactions were found in a common network induced by all three drugs. Four sets of novel genetic interactions were further investigated at the molecular level. We present the results of this work in chapter 3 to 6.

In chapter 3, we describe the identification of genetic interactions between *RTT109*, a histone H3 acetyltransferase, and genes encoding for components of one of the translesion synthesis polymerases (TLS) Polζ. These observations prompted us to test whether Rtt109

affects the mutagenic bypass of DNA lesions by TLS polymerase. Indeed, we found that the loss of Rtt109 causes a decrease in the rate of mutations generated by TLS polymerases after exposure of cells to UV light. Analysis of the UV-induced mutation spectra (at the *CAN1* gene) and mutation rates in strains defective for either Rtt109 or one of the TLS polymerases or both suggests that Rtt109 affects translesion synthesis driven by Polζ and Polη. Thus, we identify Rtt109 as a novel factor that regulates the mutagenic bypass of DNA lesions driven by different TLS polymerases.

In chapter 4, we found that two genes, *RUB1* and *UBC12* which encode key components of the yeast neddylation machinery, displayed strong negative interactions with numerous DNA damage checkpoint genes, including *RAD17*, *DDC1*, *RAD9* and *RAD24*, in response to CPT-induced DNA damage. Thus, we investigated a role for the neddylation machinery in DNA damage checkpoint control. We not only found that loss of neddylation leads to perturbations in cell cycle progression in the presence of CPT, but also increases genome instability in checkpoint-deficient cells. Neddylation is a process by which proteins are modified through the attachment of a peptide called Rub1. Among the prime targets are Cullin RING ubiquitin ligases (CRLs), which are responsible for the degradation of proteins involved in many cellular processes. To further explore how neddylation could affect the cellular response to CPT-induced DNA damage, we examined whether this process would target proteins involved in the DDR. Interestingly, we found that neddylation has an impact on the steady state levels of non-CRLs proteins such as Mms22 and Nhp10, which been shown to be involved in cell cycle control and DNA repair. Thus, we propose that the neddylation by regulating the steady state levels of distinct DDR factors affects DNA repair, cell cycle control and genome stability.

Appendix

We showed that the genetic interactions induced by each drug are very specific (chapter 2). This suggested that a unique set of DDR pathways is triggered depending on the type of DNA damage that is induced. However, we also found a significant number of changes in the genetic interactions that were induced by at least two of the genotoxic compounds. We called this overlap between the genetic networks the common DDR network. The common network not only included several known DDR factors, but also revealed unanticipated and poorly characterized genes such as *IRC21*. Chapter 5 describes the identification and characterization of Irc21 as a novel factor involved in the response to DNA damage. We showed that deletion of *IRC21* not only suppresses the sensitivity of checkpoint mutants to CPT and MMS, but also their genome instability and their cell cycle and DNA repair defects. In addition, we observed that loss of *IRC21* renders cells hypersensitive to MMS when combined with the TOR inhibitor rapamycin, a compound that can lead to increased and decreased abundance of proteins (via the autophagy pathway), including factors involved in the DDR. This suggests that Irc21 may affect the DDR by regulating the steady state levels of distinct DDR proteins.

Chapter 6 reports on the negative interaction (MMS and CPT-induced) between the DNA repair gene *SAE2* and a protein phosphatase encoding gene, *PPH3*. We attempted to understand how this DNA repair factor and protein phosphatase would interact to coordinate the signaling and repair of DNA damage. We found that cells deficient for *SAE2* and *PPH3* display severe checkpoint defects in response to DNA damage. Moreover, the repair of DNA damage induced by the alkylating agent MMS was impaired in these cells, whereas repair of

nuclease-induced DNA double stranded breaks remained unaffected when compared to that in the repair-deficient *sae2*Δ cells. We propose that the co-operation between Sae2 and Pph3 is important for efficient DNA repair and checkpoint activation in response to replication fork-associated damage induced by MMS, but is dispensable for DSB repair.

In conclusion, our multi-conditional genetic interactions screen has proven to be an extremly powerful method to unravel the interconnections between factors or signaling pathways that regulate cellular responses to various types of DNA damage. In combination with functional studies of these genetic interactions, mechanistic insight into the role of novel factors in the orchestration of the DDR can be obtained. Given that the DDR is highly conserved from yeast to man we anticipate that our genetic interaction map will also inform on the human DDR and its associated diseases.

Appendix