Summary

Higher eukaryotes have evolved mechanistically distinct repair pathways to remove DSBs from the genome: c-NHEJ, HRR and alt-EJ. The probability to faithfully repair the DSB is strongly dependent on the utilized repair pathway and HRR is the only known error-free repair pathway, whereas c-NHEJ and especially alt-EJ are known to induce sequence alterations or even translocations. How the repair pathway selection is regulated in the cell is currently under investigation and in the present work we specifically focused on HRR activity regulation and on chromatin structure as a parameter influencing repair pathway choice.

DSB repair was systematically evaluated by analyzing the formation of different IRIF in heterochromatic and euchromatic chromatin regions and we concentrated our analysis on late S and G_2 phase cells to track HRR activity. The results of γH2AX foci formation demonstrated clear incidence in EC and HC regions. To specifically visualize DNA end resection and HRR activity, we examined formation of RPA and Rad51 foci. We demonstrated that the choice towards HRR is not regulated by chromatin structure. Indeed, we observed proportional distribution of RPA and Rad51 foci in EC and HC regions.

Moreover, the numbers of Rad51 foci saturated with increasing radiation dose independently of chromatin condensation status, supporting recent findings from our laboratory revealing a saturation of HRR with increasing radiation dose. However, RPA foci in HC and EC regions increased almost linearly, demonstrating active resection at high doses, which suggests a repair pathway switch towards error-prone repair mechanisms. The dose response curves of 53BP1 foci show a similar saturation as Rad51 foci and we observed persistence of 53BP1 foci after high radiation doses. These findings suggest a regulating role of 53BP1 in the process of HRR saturation.

In order to study DSB repair under altered chromatin condensation conditions, we applied hypertonic or hypotonic treatments. Hypertonic treatment causes an increase in chromatin condensation and impaired DSB repair. Although we detected enlarged γH2AX foci formation in hypertonically treated cells, the formation of pATM, 53BP1, RPA and Rad51 foci was almost completely suppressed. Treatment in hypotonic medium, on the other hand relaxed chromatin and was better tolerated during DSB repair. This was shown by the almost unaltered formation of 53BP1, pATM and RPA foci. Unexpectedly, γH2AX foci formation was suppressed in hypotonically treated cells, which indicates that 53BP1 and RPA are able to accumulate at the break site without extensive phosphorylation of H2AX. With the help of repair reporter assays we detected the highest repair reduction with the HRR reporter assay

under hypertonic and hypotonic conditions, demonstrating that HRR is highly sensitive to changes in chromatin structure. Notably, inhibition of the histone methyltransferase SUV39H1 with chaetocin also strongly suppressed HRR.

Thus, the results obtained in the present thesis strongly support an HRR saturation with increasing radiation dose and demonstrate a regulatory role of 53BP1 in this process. Moreover, chromatin modifications were successfully established as key regulatory parameters of HRR.