SUMMARY

Our DNA is continuously exposed to hazardous DNA damaging agents such as UV and ionizing radiation as well as metabolic products. However, our cells are very well equipped to repair the induced damage. Depending on the type of DNA damage, different repair pathways can be engaged. DNA double strand breaks (DSBs) are among the most toxic types of DNA damage and can be either repaired by error prone Non Homologous End Joining (NHEJ) or error free Homologous Recombination (HR).

DNA is embedded in a structure called chromatin, which poses a barrier for the repair machinery to reach the damage site. The chromatin can be opened up by chromatin remodelers to provide access for DNA damage response (DDR) proteins. Yet, there are still many questions to be answered about the way chromatin remodelers help to increase the efficiency of the DSB response.

In this thesis I have explored the role of chromatin remodelers in the DSB response. Additionally, I have underscored the importance of known DSB repair factors in DSB repair.

In Chapter 2, we discovered a new role for the chromatin remodeler CHD4 in the DSB response. CHD4 is the ATPase subunit of the NuRD chromatin remodeling complex. We showed that CHD4 and other proteins belonging to the complex are recruited to sites of DNA damage, which suggests that NuRD plays a direct role in the DSB response. Indeed, we found that CHD4 is required for protein ubiquitylation by the E3 ubiquitin ligases RNF8/RNF168 at ionizing radiation induced foci (IRIF) and the subsequent recruitment of RNF168 and BRCA1. Depletion of CHD4 appeared to delay repair of DSBs, indicating that CHD4 modulates DSB repair.

Chapter 3 describes a novel role for the chromatin remodeler SMARCA5/SNF2h in the DSB response. SMARCA5 acts directly at DSB sites where it interacts with RNF168 to promote ubiquitylation. The recruitment of SMARCA5 to sites of DNA damage and the interaction with RNF168 is dependent on poly(ADP-ribosyl)ation. Interestingly, SMARCA5 is involved in DSB repair by both HR and NHEJ. Our study suggests that SMARCA5 specifically interacts with RNF168 to promote ubiquitylation at DSB sites. Additionally, SMARCA5 might have other functions in DSB repair that are not related to RNF168.

In Chapter 4 we show that the chromatin remodeler CHD2 a family member of CHD4, also plays a role in the repair of DSBs. More precisely, CHD2 is involved in NHEJ. Additionally, we suggest that CHD2 binds to poly(ADP-ribose) (PAR) chains through a putative PAR binding domain. Indeed, the quick and transient recruitment of CHD2 was fully dependent on the activity of poly(ADP-ribose)polymerase (PARP, which catalyzes the formation of PAR chains) as in the presence of PARP inhibitor CHD2 did not accumulate at sites of DSBs. It is conceivably that PAR may act as an anchor to direct proteins to DSBs, but in fact the precise function of PAR and PARP in the DSB response is not known.

In addition to chromatin remodelers, we also investigated Rad51C, a Rad51 paralog that interacts with Rad51 in HR (Chapter 5). To further study the function of Rad51C we started to develop a Rad51C knock-out mouse (Chapter 5). However, Rad51C$^{-/-}$ mice were not viable beyond 8 days after gestation indicating that Rad51C

is not only important for DSB repair, but also for mouse development. Although Rad51C heterozygote mice did not have any apparent phenotype, we observed that Rad51 heterozygote ES cells were more sensitive to DNA damage compared to wild type cells, which suggests impaired DSB processing.

The data described in this thesis together with recently published data, suggests a model of spatiotemporal regulation of the DNA damage response in which several chromatin remodelers interact specifically with DDR proteins. This model explains why a variety of chromatin remodelers is involved in regulation of the DDR.