Abstract

The Mre11-Rad50-Nbs1 (MRN) protein complex is a well known sensor of DNA damage, functioning in signaling pathways that activate cell cycle checkpoints. The function of Mre11 is also known to be important for homologous recombination repair (HRR), and recent studies have shown that this protein may function both in HRR, as well as in the classical pathway of non homologous end joining that utilizes in addition to DNA-PK (D-NHEJ) also Ku and the Ligase IV/XRCC4 complex. Indeed, in yeast, in addition to Ku and Ligase IV homologs, also the MRN homologs are implicated in NHEJ. The Ku70/80 heterodimer is among the first proteins that recognize and bind to DNA ends. The crystal structure of Ku reveals an asymmetric ring conformation allowing the threading of the DNA and possibly facilitating rejoining. After completion of end joining, however, the protein will remain trapped on the DNA. This mode of action generates the question as to how trapped Ku is released from the DNA, and as to whether trapped protein serves somehow to the development of a full-blown DNA damage response. Hitherto, only one report suggests a ubiquitin-dependent removal and degradation of Ku80 from DNA, which is independent of the completion of NHEJ. There are no reports published to date investigating a functional role for the trapped Ku protein in the ensuing DNA damage response (DDR) signalling. The present thesis tests the hypothesis that trapped Ku contributes to DDR signalling by somehow facilitating the recruitment of the MRN complex to the site of the DSB. In support of this hypothesis, we demonstrate here that the constitutive interaction between Mre11 and Ku is enhanced after exposure to IR and that this enhancement is dose dependent. The interaction between Mre11 and Ku is studied by immunoprecipitation (IP) from nuclear extracts using an anti-Ku70 antibody while detecting Mre11 by Western blotting. IP studies are carried out using a human alveolar basal epithelium carcinoma cell line (A549), as well as CHO cells and a Ku80 deficient mutant (*xrs6*). As expected, Ku deficient cells show no detectable interaction between Mre11 and Ku70 and validate thus the specificity of the assay. An interaction between Mre11 and Ku is clearly visible in A549 cells and is markedly enhanced after exposure to 2 and 4Gy of X-rays. The interaction is resistant to EtBr and RNAase suggesting that it is not mediated by DNA or RNA. Rad50 and Nbs1 are also co-precipitated with Mre11, suggesting that the entire MRN complex is involved in the interaction. Notably, in addition to MRN, other DNA

damage sensors such as PARP-1 and ATM are part of the complex and are co-precipitated. The results point to intriguing interactions between Ku and signaling/repair proteins such as the MRN complex that warrant further investigation. The kinetics of Mre11 interaction with Ku after exposure to 4 and 8 Gy X-rays showed a weak Mre11 signal at 10 min after irradiation, but a strong signal 1-2 h after irradiation. When this interaction was tested in cells defective in PIKK family proteins, DNA-PKcs and ATM, it was found that DNA-PKcs defective cells show no Ku-MRN interaction following exposure to radiation. Treatment of extracts with bacterial alkaline phosphatase led to a decrease in the dose dependent interaction between Mre11 and Ku, while treatment with sodium ortho-vanadate and sodium azide (inhibitors of protein tyrosine phosphatases) led to an decrease in overall interaction but IR-dependent aspect remains unchanged.

The second part of my thesis focuses on the activity of PARP-1, which is a putative component of the backup pathway of non-homologous end joining (B-NHEJ), where it may function together with DNA Ligase III and histone H1. Goal of this part of the work was to examine possible interactions between Mre11 and PARP-1 and thus to establish a possible cross talk between D-NHEJ and B-NHEJ. The results show a marked increase in PARP-1 activity with increasing concentration of histone H1.