Summary

In all biological processes in each living cell, proteins perform a key function. The blueprint of each protein is stored in the genetic material or DNA. It is crucial for a cell to protect its DNA in order to keep performing its function. The most critical moment in which the DNA is most viable occurs during the S-phase of the cell, when the DNA is duplicated. To ensure correct duplication, the cell has developed specialized proteins, each performing a specific task to complete DNA synthesis. To initiate and regulate new DNA synthesis, the five protein complex Replication Factor C (RFC) loads the ring shaped PCNA onto the DNA. On its turn, PCNA is an important platform for polymerases, to efficiently synthesize new DNA.

DNA polymerases utilize the specific pairing of nucleotides. Any deviation from this specific or so-called Watson-Crick pairing significantly decreases the ability of the polymerase to synthesize DNA. This ensures a very low error-rate during DNA synthesis. However, in the situation of a damaged nucleotide, the polymerase stalls. To overcome this problem, the cell has developed specialized polymerases that can insert a nucleotide opposite damaged DNA. This process, called DNA Translesion Synthesis, circumvents damaged DNA and enables DNA to be completely duplicated. The downside is, however, the high possibility of introducing a point mutation.

This thesis focuses on Rev1, one of these specialized DNA Translesion Synthesis polymerases. Rev1 is unique in the circumvention of DNA damage by performing a organizing role besides its role as a polymerase. Recently, it has been shown that a part of the Rev1 protein, consisting of a BRCT domain, is essential for this organizing role. The exact function of this BRCT region, however, remains unknown. The biochemical characterization is the primary subject of this thesis. Furthermore, this thesis focuses on the biological role of the BRCT region of Rfc1, which is closely related to the Rev1 BRCT region.
Chapter II focuses on the interaction between the Rev1 BRCT region and DNA. By producing a different amount of protein fragments and analysing their DNA binding properties, the part of the protein that is essential for DNA binding can be determined. Furthermore, by using different DNA substrates the optimal DNA substrate can be determined. It appears that DNA binding does not only require the BRCT domain itself, but the entire BRCT region. Furthermore, the protein specifically binds 5’ phosphorylated primer terminus.

By replacing specific amino acids using site-directed mutagenesis several amino acid residues of the Rev1 BRCT region were identified to be involved in DNA binding. Since DNA binding by the Rev1 BRCT region resembles DNA binding by the Rfc1 BRCT region, a model structure of DNA bound to the Rev1 BRCT region can be designed, based upon the elucidated structure of the Rfc1 BRCT region bound to DNA.

Since the previous chapter analysed the DNA binding properties of the BRCT region by itself, Chapter IV discusses the DNA binding properties of the complete Rev1 protein. Here it is demonstrated that the Rev1 BRCT region specifically binds to the 5’ phosphorylated primer terminus, independent from other parts of the protein, while the polymerase domain appears to solely and independently bind the 3’ primer terminus. Interestingly, binding of both primer termini appears to be cooperative, suggesting that in a biological situation, Rev1 could possibly perform a bridging role between signaling events at both termini.

By knocking out complete genes, the function of the gene can be determined. When both a gene with a yet unknown function, and a gene with a known function are knocked out, the possible shared biological relevance can be determined. Chapter V determines the possible role of the Rfc1 BRCT region in different DNA repair or replication pathways. It appears that RFC contributes to the efficiency of a number
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of these pathways. Possibly, Rfc1 performs an organizing role in these processes, which depends on the BRCT region of Rfc1.

To completely understand the contribution of the DNA binding properties to the biological function of Rev1, the data presented in this thesis is not sufficient. However, recently the BRCT region of Rev1 has been found to be essential in different pathways concerning DNA damage repair or avoidance. Chapter VI discusses the possible models to which the DNA binding properties of the Rev1 BRCT region can contribute. Here, it is obvious that the biological role of Rev1 goes beyond DNA Translesion synthesis. The DNA binding properties of the Rev1 BRCT region appear to correspond nicely with the new gained insights in the repair or circumvention of DNA damage.