High fidelity DNA replication and repair: new structures and mechanisms using cryogenic electron microscopy

Borsellini, A.

Appendix

Summary

Introduction contains a general overview of the research topics discussed in this thesis. First, it provides a background on molecular mechanisms involved in DNA replication and DNA mismatch repair. This is followed by a description of bacterial DNA polymerases as potential target for the development of new antibiotics. Finally, a section on the technical challenges of cryo-electron microscopy and the introduction of a new method to overcome these.

Chapter 1 addresses a fundamental question in DNA mismatch repair, which is how ATP binding and hydrolysis drive the conformational changes in MutS that are needed for the mismatch repair cascade. It describes four cryo-EM structures of *E. coli* MutS during sequential stages of the ATP hydrolysis cycle. The structures in combination with biochemical assays describe the modulation of the ATPase activity of MutS exerted by the DNA.

Chapter 2 focuses on the final stages of the DNA mismatch repair pathway, which are the resection and subsequent resynthesis of the mismatch containing strand. It describes a novel function of the DNA mismatch repair protein MutL which is to block DNA polymerase access to 3' resected DNA ends. These findings help to understand the interplay between mismatch repair and DNA replication during the final steps of the repair cascade.

Chapter 3 presents an example of how DNA polymerases can be targeted for the development of novel antibiotics against *Mycobacterium tuberculosis* (Mtb). We demonstrate that nargenicin, a natural product that targets the replicative DNA polymerase of *Staphylococcus aureus*, is a bactericidal genotoxin that induces a DNA damage response and inhibits growth in *Mycobacterium tuberculosis* through binding between the polymerase and DNA.

Chapter 4 describes a new instrument named the Puffalot, developed for the preparation of cryo-EM grids, which aims to improve the reliability of cryo-EM sample preparation. In addition to this, the chapter discusses how the new plunge freezer can be used to perform time-resolved cryo-EM experiments and study proteins conformational changes that occur in the millisecond time scale.

Discussion provides a summary of the scientific findings described in this thesis in light of the published literature as well as an overview of the future directions and perspectives.