Single-molecule studies of bacterial DNA replication and translesion synthesis

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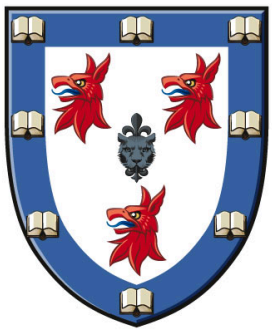

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SUMMARY

Faithful replication of genomic DNA is crucial for the survival of a cell. In order to achieve high-level accuracy in copying its genome, all cells employ replicative DNA polymerases that have intrinsic high fidelity. When an error occurs on the template DNA strand, in the form of lesions caused by diverse chemicals, reactive oxygen species, or UV light, the high-fidelity replicative DNA polymerases are stalled. To bypass these replication blocks, cells harbor multiple specialized translesion DNA polymerases that are error-prone and therefore able to accommodate the lesions and continue DNA synthesis. As a result of their low fidelity, the translesion polymerases are associated with increased mutagenesis, drug resistance, and cancer. Therefore, the access of the translesion polymerases to DNA needs to be tightly controlled, but how this is achieved has been the subject of debate.

This Thesis presents the development of a co-localization single-molecule spectroscopy (CoSMoS) method to directly visualize the loading of the *Escherichia coli* replicative polymerase on DNA, as well as the exchange between the replicative polymerase and the translesion polymerases Pol II and Pol IV. In contrast to the toolbelt model for the exchange between the polymerases, this work shows that the translesion polymerases Pol II and Pol IV do not form a stable complex with the replicative polymerase Pol IIIα on the β-clamp. Furthermore, we find that the sequential activities of the replication proteins: clamp loader, clamp, and Pol IIIα, are highly organized while the exchange with the translesion polymerases is disordered. This exchange is not determined by lesion-recognition but instead a concentration-dependent competition between the replicative and translesion polymerases for the hydrophobic groove on the surface of the β-clamp. Hence, our results provide a unique insight into the temporal organization of events in DNA replication and translesion synthesis.

DECLARATION

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit of 60,000 words as set by the Biology Degree Committee.

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