ABSTRACT

The DNA replication is the most fundamental process occurring in the cell and the molecular basis for replisome activity has been extensively investigated for several decades. However it is not clear what the exact mechanism for *de novo* assembly of the replication complex at the replication origin is, nor how the directionality of replication is determined. DNA synthesis of prokaryotic and eukaryotic replicons requires the coordinated action of several enzymes. These enzymes cooperate to form specific nucleoprotein complexes during the course of DNA replication. The formation of the initial complex is a result of a replication initiation protein (Rep) or Origin Recognition Complex binding to double-stranded DNA within the origin of DNA replication initiation. This interaction of the replication initiators with DNA results in origin opening *i.e.* destabilization of double-stranded DNA helix. Origin opening provides single stranded DNA for helicase, primase and finally polymerase. The specific motif determining interaction with β clamp subunit of *Escherichia coli* Polymerase III has been identified in plasmid Rep proteins, however, the relevance of the interaction between the β clamp and Rep proteins has not been established.

In this work, I investigated the significance of the β clamp interaction with the TrfA replication initiator in the process of RK2 plasmid DNA replication initiation. With the use of the plasmid RK2 replicon, I analyzed the protein interactions required for *E. coli* Polymerase III holoenzyme association at the replication origin. The investigations revealed that in *E. coli*, replisome formation at the plasmid origin involves interactions of the plasmid replication initiation protein, TrfA, with both the polymerase β and α subunits. In the presence of other replication proteins, including DnaA, helicase, primase and the clamp loader, TrfA interaction with the β clamp contributes to the formation of the β clamp nucleoprotein complex on origin DNA. By reconstituting *in vitro* the replication reaction on single-stranded DNA (ssDNA) templates we demonstrate that TrfA interaction with the β clamp and sequence specific TrfA interaction with one strand of the plasmid origin DNA Unwinding Element (DUE), contributes to strand specific replisome assembly. Wild-type TrfA but not the TrfA QLSLF mutant (which does not interact with the β) in the presence of primase, helicase and Polymerase III core, clamp loader and β clamp initiates DNA synthesis on ssDNA templates containing 13-mers of the bottom but not the top strand of DUE. I also uncovered interactions significant in anchoring polymerase at the bottom strand DUE of the plasmid replication origin. These results bring new insights of how the directionality of DNA replication is determined.