6. Summary

The DnaA protein is the central initiator protein of the bacterial replication. DnaA binds specifically to its recognition sequence within *oriC*. This leads to a specialiced nucleoprotein complex (initial complex) and results in the local unwinding of the DNA in the left half of *oriC*. DnaA recruits and loads the helicase onto the unwound region. Establishing of a bidirectional replication requires loading of two helicases.

The aim of the work was the identification and characterization of interaction domain(s) between the initiator protein DnaA and the replicative helicase DnaB as well as studies of the DnaA mediated loading of the helicase at *oriC*.

Using the Cos-assay I identified a region of DnaB (aa 203-213) that was able to suppress the cold-sensitive phenotype of the *dnaA219*(Cos) strain in context with adjacent protein regions upstream (DnaB[154-206]) or downstream (DnaB203-471]), respectively. The inhibition of initiation, measured as growth at 30 °C, is presumably due to a direct interaction between DnaA and DnaB.

An *in vivo* assay was used to identify regions of DnaA involved in helicase loading. In this assay the loading of the helicase is uncoupled from other steps, especially from the unwinding of the DNA. DnaA domains 1 (aa 24-86), 3 (aa 135-373) and 4 (aa 374-467) are essential for the loading of the helicase. Mutations within domain 3 that result in a decreased nucleotide binding affinity but do not affect the structure of the domain could load the helicase.

Solid-phase Protein Binding Assays show that DnaA and DnaB interact directly. The interaction requires no DNA contact. Both proteins have two interaction domains. The DnaA N-terminus (aa 24-86) interacts with DnaB (aa 203-213) and DnaA domain 3 (aa 130-149) with the DnaB α-fragment. The primary interaction domains of the proteins are the DnaA N-terminus (aa 24-86) and DnaB (aa 203-213). DnaA domain 4 is not involved in a direct interaction with the helicase.

The formation of a specific nucleoprotein complex at *oriC* of *E. coli* and the origin of pSC101 requires a DnaA-DnaA interaction. The self-interaction of DnaA happens via the N-terminus (aa 1-77). The DnaA-DnaA interaction domain overlaps with the DnaA-DnaB interaction domain. DnaA domains 1 and 4 are necessary and sufficient to promote pSC101 replication. The DnaA homooligomerisation via the N-terminus is a long-range interaction.

A substrate that mimics the unwound region was used to simulate the loading of the helicase onto *oriC*. A single DnaA is sufficient to load the helicase onto an

adjacent ssDNA. DnaA bound to the DnaA box R1 in *oriC* loads the helicase onto the lower ssDNA strand. The loading of the helicase is independent of the sequence of the single-stranded DNA. The helicase for the other DNA strand is loaded by another DnaA protein, presumably the DnaA box R2.