Abstract

Bacterial TA systems are chromosomally or plasmid-encoded gene pairs found in free-living bacteria that often aid in survival during environmental and chemical stress. They become active during stress, but their role in regulating the cellular process is unclear to date. Also, parts of the same TA system in different bacteria function differently, which makes it challenging to study and find similarities between them in other bacterial species. In this thesis, we have studied the type II TA system- the HigBA TA system of *Caulobacter crescentus*. *Caulobacter* undergoes dimorphic cell division giving rise to two morphologically different daughter cells with different replicative potentials, making it easy to study its cell-cycle. While investigating the role of HigBA TA system of *Caulobacter,* which is a DNA damage-inducible TA system we found the HigBA TA system, to be a three-component system instead of two-component system (the toxin and the antitoxin). The third component, named as HigC in the thesis, is a transcriptional regulator. It was seen that HigC auto-regulated the TA system and also worked independently of it.

While investigating the role of the toxin HigB in the regulation of the cell cycle through the master regulator CtrA, the involvement of HigC was also observed which made the study more interesting as it would be a unique role for a TA- associated transcription factor that has not been observed before. It was also observed that during an antibiotic (Ciprofloxacin) stress HigB and HigC affected the cell cycle regulation inversely. While HigB reduced the expression of the CtrA regulated promoters HigC increased the expression of the CtrA regulated promoters. It was previously suggested that the important function of TA system was persister formation; therefore, we have also investigated the role of HigBA and HigC in persister formation in response to ciprofloxacin, but neither HigBA nor HigC showed any changes during persister formation. Thus, we proposed that the HigBA TA system as an exclusively SOS induced three-component TA system whose role in an SOS response is to regulate cell cycle-dependent gene expression, which makes it a unique TA system. It is also seen that can HigBA regulates the cell cycle network through CtrA, which is unique for a TA system. All the above results could be found in the manuscript attached to this thesis. In Chapter2 of this thesis, we had also successfully purified the TA system proteins HigA, LexA, HigC by His tag Ni affinity chromatography and

optimized processes to purify toxin HigB protein by the IMPACT ™ purification method by NEB. All these purified proteins along with HigB which, when successfully purified, could be used in future to investigate the mechanism behind the roles of the HigBA and HigC TA system of *Caulobacter crescentus* by in-vitro analysis.