Summary

Anabaena PCC 7120 is a heterocystous filamentous cyanobacterium, capable of diazotrophy. In the absence of combined nitrogen source, ~10% of the vegetative cells undergo terminal differentiation in to heterocyst which provides suitable anaerobic condition for the activity of oxygen-sensitive nitrogenase enzyme system. Late stages of the heterocyst maturation involve three developmentally regulated gene rearrangements. In this organism, three genetic rearrangements occur in *nifD, fdxN* and *hupL* genes, called *nifD* element, *fdxN* element and *hupL* element, and their sizes are 11,278 bp, 59,428 bp and 9,419 bp, respectively. Excision of the element is necessary for the functionality of the encoded protein. *xisA* encodes a site-specific recombinase Excisase A, involved in the excision of *nifD* element. XisA, XisF and XisC are absolutely required for the site-specific recombination and they do not show cross reactivity. XisA and XisC belong to integrase family of tyrosine recombinase while the XisF belongs to resolvase family. All tyrosine recombinases except Cre and FLP, requires additional DNA sequences, other than cross-over site to carry out recombination. XisA and XisC contain a conserved *int* domain at their C-terminal region, while XisF shows conserved domain similar to SPOIV C.

Excision events of the *nifD* element have been studied in *E. coli* using pMX25 plasmid, which contains entire 11kb element, on the basis of blue-white selection. Earlier studies in the laboratory established that XisA possess endonuclease activity in addition to recombinase activity. The relationship between the structural domains of the XisA and these two activities are not known as well its expression and regulation. Thus, objectives of the present study were to characterize XisA as restriction endonuclease, determine the role of N-terminal and C- terminal region of XisA, and study its promoter activity using GFP reporter gene.

Chapter 2 focuses on the role of accessory sequences in the recombination. Recombination region of XisA contains an 8 bp inverted repeat TTTTTGTG nearby to the 11 bp recombination site CGGAGTAATCC. To study the involvement of the inverted repeat, different substrate plasmids were constructed and monitored for the rearrangement in presence of XisA and NifA pMX32 containing 11 bp, 8 bp along with NtcA binding sites showed excision of *nifD* element. Substrate plasmids of 11 bp and 11 bp + 8 bp sequences did not show excision. Thus, besides the 11 bp target site accessory sequences are required to carry out excision of *nifD* element.

Chapter 3 is centered around characterization of XisA as restriction endonuclease. Different substrate plasmids were constructed with one base pair mutation in the 11 bp target site. Substrate plasmids were constructed with one or two copies of the target site. All the substrate plasmids were checked for the specific loss of target site containing plasmid in presence of XisA and XisA along with NifA. All mutations of the target site resulted in the loss of endonuclease activity except one involving change of A to C at 3rd position. Substrate plasmid with two target site showed more endonuclease activity as compared to with one target site. Based on these results, XisA is suggested to belong to Type IIE type of restriction endonuclease with probable target site GGN$_5$TCC.

Chapter 4 describes various methods to study the fate of *nifD* element after its excision. pMX25 containing entire 11 kb element with two copies 11 bp target sites, pKK1 containing one copy of 11 bp target sites and pMC71A containing *nifA* gene were transformed in JM101 *E. coli* strain and monitored for the intermolecular/intramolecular recombination based on antibiotic resistance marker and blue-white selection. *nifD* element was found to undergo intramolecular recombination resulting in its excision at a frequency of ~55% which was comparable to earlier studies, while no intermolecular recombination was detected.

Chapter 5 describes the functional characterization of XisA and XisC domains. In order to determine the role of N-terminal and C-terminal regions of XisA and XisC, C-terminal and N-terminal domains of these enzymes were cloned separately. Chimeric recombinase containing N-terminal of XisA and C-terminal of XisC was also constructed. All these plasmids were monitored for the recombination of XisA and XisC substrate plasmids, pMX25 and pAM1500 respectively as well as for the endonuclease activity. N-terminal of XisA did not show any activity, while the C-terminal alone was found to be sufficient for the recombinase as well as endonuclease activity with specificity similar to XisA. XisC C-terminal region did not show any rearrangement and endonuclease activities. Chimeric recombinase showed rearrangement of XisC substrate plasmid pAM500. Hence, XisA and XisC are functionally different even though they show very good sequence similarity. XisA does not require N-terminal region while XisC does require and it does not show any endonuclease activity.

Chapter 6 deals with study of expression of XisA promoter using GFP reporter assay. To study the regulation of *xisA* expression, its promoter was fused with GFP reporter gene and monitored the fluorescence under different growth conditions in *E. coli*. In *E. coli*, *xisA* expression was higher in stationary phase as compared to log phase in both Luria broth and M9 minimal medium. But in M9 minimal medium, higher promoter activity was found as compared to LB medium. Presence of NtcA and NifA increased the *xisA* promoter activity. These expression study results were similar to the earlier studies with β-galactosidase reporter gene. PxisA::gfp was transformed in *Anabaena* PCC 7120 for further characterization.

In conclusion, the work presented here shows various functional aspects of XisA. Besides, a recombinase, it also showed restriction endonuclease activity similar to type II E enzymes. Functionality studies of XisA and XisC Domains showed that even though they share high sequence similarity, structurally and functionally, different. C-terninal of XisA alone is capable of recombination.

GFP reporter assay to study the *xisA* promoter activity has been done in *E. coli* and transformed in *Anabaena* PCC 7120 which can be used to study the same under different physiological conditions.