SUMMARY

Anabaena PCC 7120 is a heterocystous cyanobacterium which fixes nitrogen in specialized cells, heterocysts, which develop in a semi-regular pattern in the filament in the absence of combined nitrogen in the medium. *nifHDK* operon encoding nitrogenase enzyme is interrupted by a 11 kb element, known as *nifD* element, and is excised during heterocyst development by excisase A (*xisA)* which is encoded within the element. This is a site-specific recombination process occurring at the 11 base pair direct repeats present at the ends of the *nifD* element. The entire 11kb *nifD* element along with Mud::*lacZ* containing β-galactosidase gene was subcloned in to the *E. coli* vector (pMX25) and the excision events were monitored in *E. coli* using Blue/White colony assay. *E. coli* containing the unrearranged pMX25 plasmid forms blue colony on X-Gal indicator plates whereas the *E. coli* containing the rearranged (pMXΔ25) plasmid shows white colony on the indicator plate. Earlier studies have shown that excision of this element can take place even in heterologous system of *E. coli* but occurs at very low frequency (0.3%). The restriction endonuclease digestion pattern showed that the rearrangement in *E. coli* is same as in *Anabaena*. The objective of the present work was to find out factors that could increase the excision frequency, increase the expression of *xisA* in *E. coli* and to monitor the expression of *xisA* gene in *E. coli*.

Chapter 2 describes the effect of medium, growth conditions and genetic background of *E. coli* strains on the excision of *nifD* element. In *E. coli* DH5α, the excision increases to about 1% after 24h of growth in Luria broth whereas only 0.3-0.45% was found after overnight growth. The excision further increases to 2% in M9 minimal medium. Interestingly, excision increases by about 4 fold in *E. coli* JM101 in M9 minimal medium. RecA protein in *E. coli* JM101 has been shown to be responsible for the increase in the excision as compared to *E. coli* DH5α, by constructing *recA* mutant of *E. coli* JM101 using P1 transduction and comparing it with isogenic *recA*$^+$ strain of *E. coli* JM101. The excision frequency in *recA*$^-$ mutant was same as in DH5α and the isogenic JM101 *recA*$^+$ strain showed the excision frequency similar to that of the excision in *E. coli* JM101. Nature of the nitrogen source, anaerobicity or iron starvation did not have any effect on the excision frequency. These results suggest that *xisA* may not be repressed in *E. coli* and no additional proteins involved in the excision of *nifD* element are induced under these conditions.

Accuracy of estimation of excision frequency of *nifD* element in *E. coli* colonies containing unrearranged (pMX25) and rearranged (pMXΔ25) plasmids was verified by checking for the growth rates of *E. coli* JM101 containing pMX25 and pMXΔ25 in Luria broth and in M9 minimal medium.

No significant difference in growth rates was found in Luria broth but the growth rates were increased by two folds in the *E. coli* strain containing rearranged plasmid (pMXΔ25) when grown in M9 minimal medium. However, competition experiments with 1% *E. coli* containing rearranged plasmid (pMXΔ25) and the other cells with 99% unrearranged plasmid (pMX25) showed only 13% excision frequency indicating that in M9 minimal medium *E. coli* white colony formation by growth rate advantage is not significant and the estimation of excision frequency is fairly accurate.

Results of Chapter 3 show that role of NifA protein of *Klebsiella pneumoniae* on the excision of ***nifD*** element in *E. coli*. In Luria broth, the excision frequency was increased to 5% in *recA*$^+$ and *recA*$^-$ strains in the presence of NifA whereas in M9 minimal medium it increasesd upto 70%. Excision frequency correlates with the growth curve and reaches maximum in late exponential phase. In order to find out mechainism of NifA on the excision of *nifD* element, the *E. coli ntrA* mutant was constructed by P1 transduction. The excision was monitored in this mutant and it was found that the excision frequency was only 1% in Luria broth and 2% in M9 minimal medium after 24h of growth. Thus, there was no increase in excision frequency in the presence of NifA either in Luria broth or in M9 minimal medium after 24h of growth. This suggests that σ^{54} encoded by *ntrA*

gene may be necessary for NifA of *K. pneumoniae* to increase the *xisA* expression in *E. coli*.

Chapter 4 describes the indirect demonstration of endonuclease activity of excisase A on plasmids containing one or two 11 base pair target sites but not on other plasmids which do not have these target sites. Plasmid pMX25 and pAn207.65 show susceptibility to ampicillin and also correlates with the excision of *nifD* element *i.e.* high in minimal medium conditions in the presence of NifA. The *E. coli* JM101 containing pAn207.65 and NifA protein was very susceptible to ampicillin even in Luria broth.

Chapter 5 deals with the study of the expression of *xisA* gene promoter by constructing a β-galactosidase transcriptional fusion vector. *xisA* promoter expression was found to be higher in M9 minimal medium, less in a *recA* mutant strain, and in an *ntrA* mutant. But the promoter activity did not always correlate with excision frequency. x*isA* promoter activity was high in Luria broth in the stationary phase even in the absence of NifA protein. Under these conditions the excision was not very high. The results indicated that *ntrA* is not essential for *xisA* expression but plays a significant role in increasing the excision of *nifD* element in *E. coli*.

In conclusion, the work presented here shows that *E. coli* system has been used to increase the excision frequency of *nifD* element of *Anabaena* PCC 7120 to very high levels. Endonuclease activity of XisA has also been demonstrated. Understanding the mechanism of increasing *xisA* expression would be useful in studying the XisA protein and the mechanism of excision. The results presented here clearly demonstrate that *E. coli* can be very useful in understanding the expression of *xisA* gene and the excision of *nifD* element of *Anabaena* PCC 7120.

MECHANISM OF EXCISION OF *nifD* ELEMENT OF HETEROCYSTOUS CYANOBACTERIA *ANABAENA* sp. STRAIN PCC 7120 IN *ESCHERICHIA COLI*

SUMMARY

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