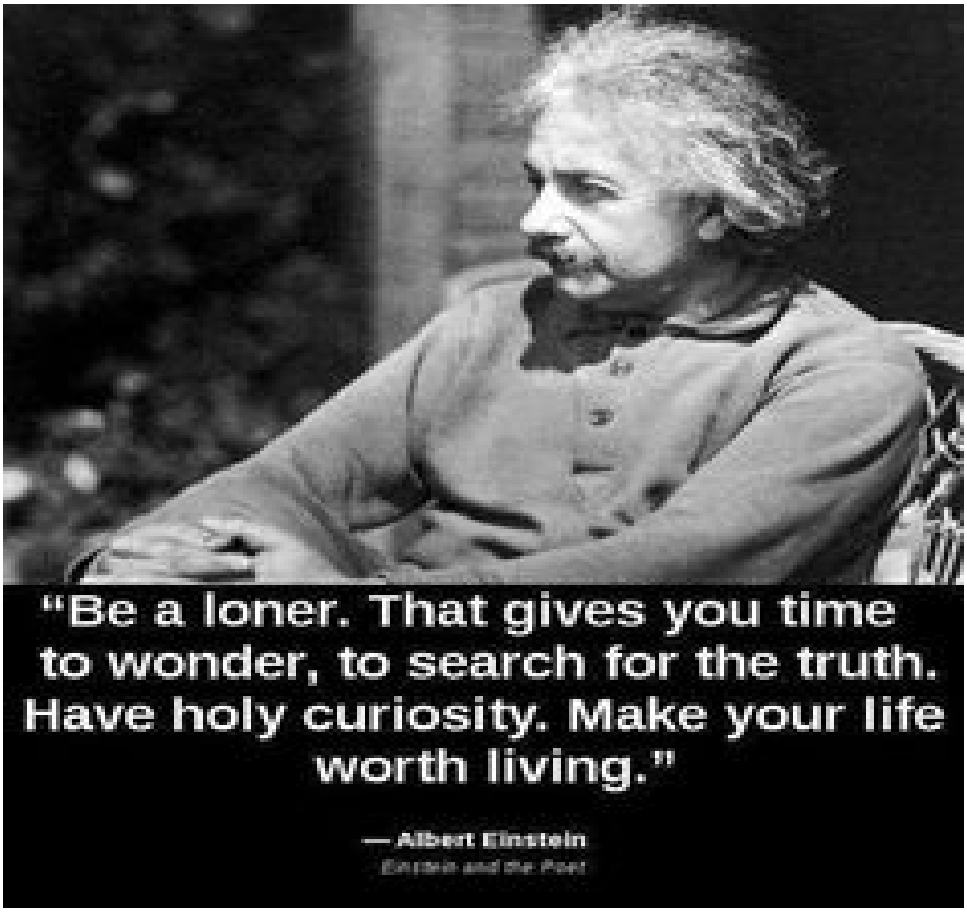
"Be a loner. That gives you time to wonder, to search for the truth. Have holy curiosity. Make your life worth living."
— Albert Einstein

Summary

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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 crossover site to carry out recombination. XisA, along with recombinase activity, also possessed endonuclease activity. However, the mechanism of such dual activity is not known. Although, *xisA* gene was cloned and sequenced long ago, neither XisA protein nor *xisA* transcript is reported till date.

Chapter 3 is centred around overexpression of *xisA* gene under T7 promoter, and purification of encoded XisA protein. Purification of 6xHis-tagged XisA protein expressed from pET28a vector was facilitated by Ni-NTA affinity purification. Expressed XisA was found to be functionally active in *E. coli* heterologous host. Purified XisA was further confirmed Protein Mass Finger Printing facilitated by MALDI-TOF analysis.

Chapter 4 dealt with developing a novel PCR based approach to monitor the activity of XisA *in vivo*. Unique substrate specific and product specific PCR primers facilitated efficient monitoring of XisA recombinase activity and addressed inaccuracy issues arising from previously reported *in vivo* methods.

Chapter 5 focussed on deciphering the regions involved in demonstrating recombinase and endonuclease activities of XisA protein. Although, N-terminal

region of XisA protein had neither of the activities (Gopit Shah thesis, 2007), it was strictly required to demonstrate recombinase function of XisA. C-terminal could stand alone exhibit endonuclease activity whereby the smallest C-terminal fragment of XisA (187 amino acid) was able to demonstrate detectable amounts of endonuclease activity. Loss of recombinase activity upon removal of N-terminal suggests its role in protein –protein interaction to form active tetramer. C-terminal retaining endonuclease activity suggests it could possess dimer forming potential in absence of N-terminal **(Fig S1)**.

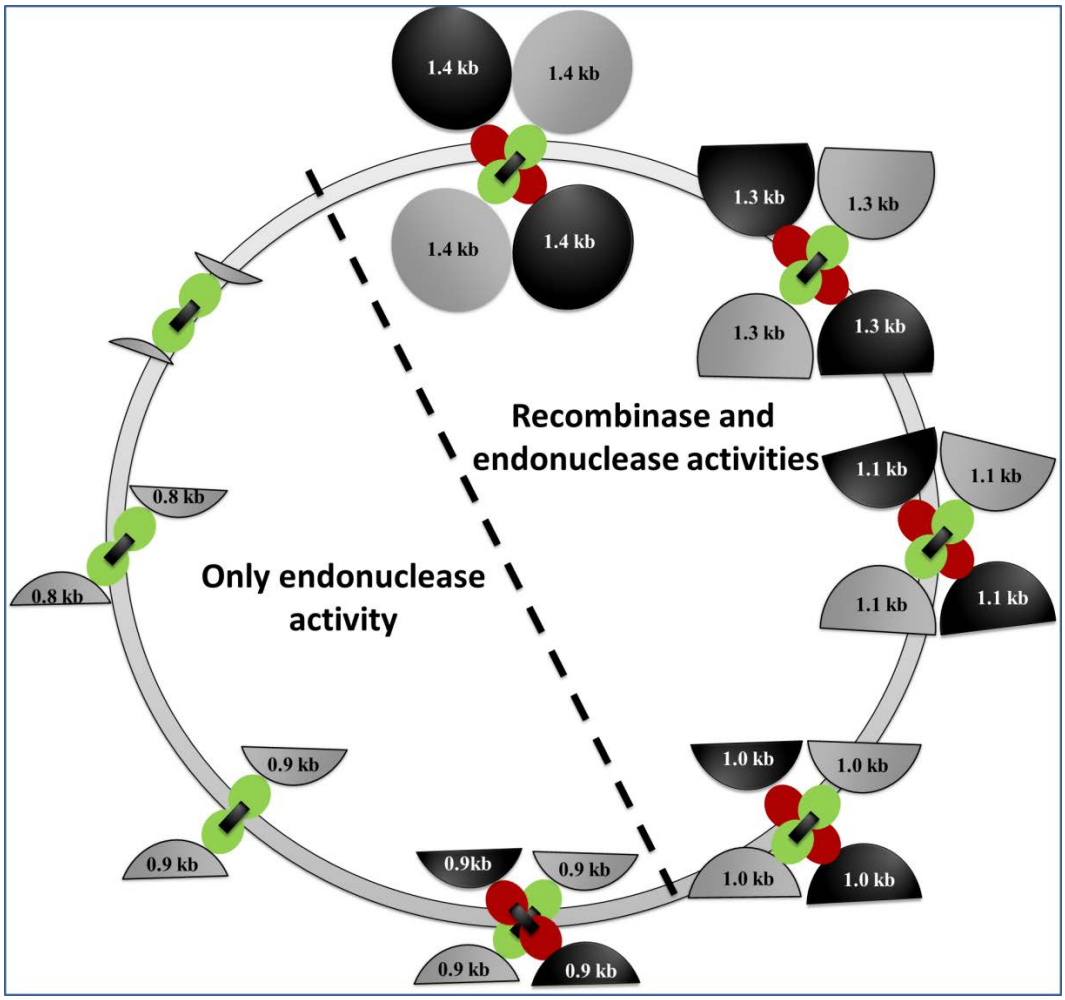

Fig. S1: Schematic representation of multimer forming potential of XisA sequential N-terminal truncation products

Chapter 6 investigated the functional role of predicted basic region of leucine zipper (bLZ) at the extreme C-terminal of XisA protein in. Deletion of bLZ led to complete loss of recombinase and endonuclease activity suggesting it could be involved in XisA protein in dimer formation **(Fig S2)**. Regain in the functionality of

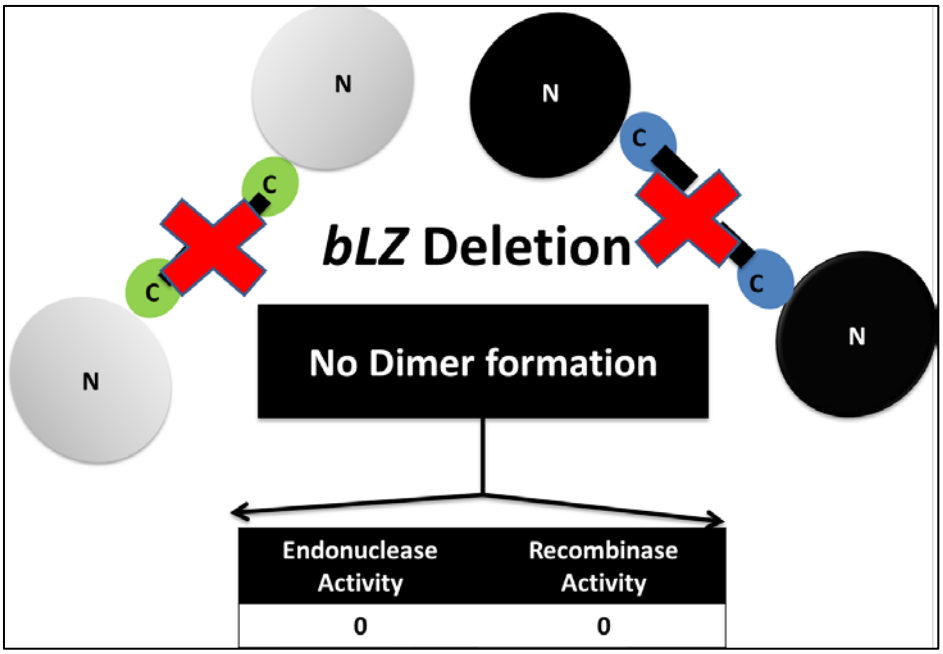

Fig. S2: schematic representation of functionality loss bLZ deleted XisA

XisA protein upon complementation with *jun leucine zipper sequence* (*j*LZ), strengthened the predicted functionality of XisA bLZ region.

Chapter 7 dealt with understanding the role of active site residues in recombinase and endonuclease activities of XisA protein. XisA protein belongs to a member of tyrosine family of recombinases characterized by **R-(X)$_{70-139}$-H/Y-XX-R-(X)$_{29-38}$-Y** catalytic tetrad . XisA exhibits 47 % identity and 65 % similarity with XisC. Despite of this fact, only XisA demonstrated endonuclease activity which is operated from its C-terminal region. XisA and XisC are distinct from most of the tyrosine recombinases in having tyrosine as second active site residue as opposed to possessing histidine. Site directed mutagenesis of primary tyrosine to histidine (Y342H) and making it similar to other recombinases, resulted in marginal elevation in recombinase and endonuclease activities of XisA1 protein. The presence of such dual activity mediated by common active site is indicative of XisA being an evolutionary intermediate between endonucleases and recombinases.

Purified N-terminal truncation products of XisA and active site mutants can be used to characterize *in vitro* endonuclease and recombinase potential of XisA.

Purified protein can be also used to determine the crustal structure of XisA, an important protein of *Anabaena* PCC 7120 nitrogen fixation.

Fig. S3 depicts a brief history of *Anabaena* PCC 7120 research right from the identification and nomenclature of cyanobacteria in 1978 by Prof. R. Y. Stanier to deciphering the dual activity mechanism of XisA in demonstrating recombinase and endonuclease activities.

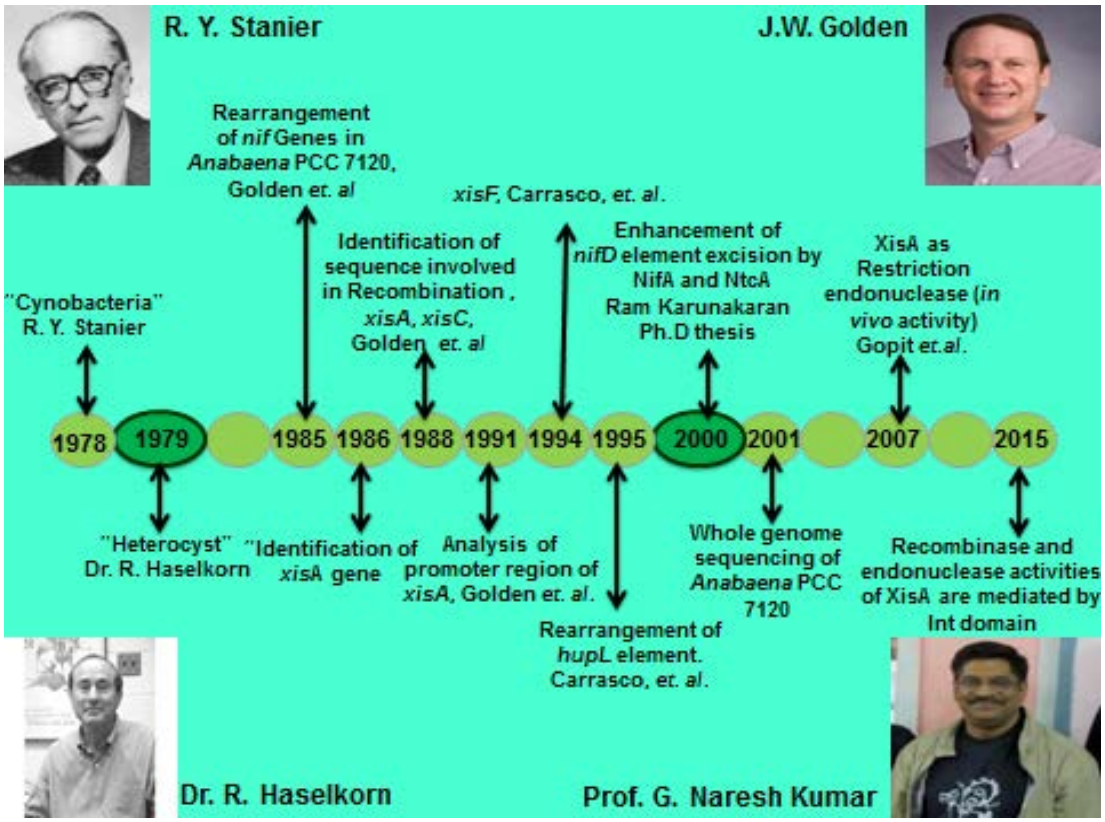

Fig. S3: A brief timeline of *Anabaena* PCC 7120 research