Chapter 5: Functional characterization of XisA and XisC domains

5.1 Introduction

Anabaena PCC 7120 is a heterocystous cyanobacteria, which undergoes terminal differentiation to form ~10% heterocyst in the filament, in the absence of combined nitrogen source. *Anabaena* PCC 7120 and some other species of cyanobacteria show developmentally regulated gene rearrangements (Golden *et al.*, 1987, Haselkorn, 1992, Carrasco *et al.*, 1994). *Anabaena* PCC 7120 genome contains three interrupting elements, which get excised out during the late stages of heterocyst maturation by site specific recombination processes. XisA, XisC and XisF are responsible for the excision of 11,278 base pair *nifD* element, 59,428 base pair *fdxN* element and 9,419 base pair *hupL* element, respectively (Kaneko *et al.*, 2001). Other cyanobacteria contain varying sizes of *nifD* element e.g. *nifD* element of *Nostoc punctiforme* is of 24 kb while the *Anabaena* strain ATCC 33047 contains a 4kb *nifD* element (Henson *et al.*,2005). All the three site specific recombination events occur simultaneously prior to nitrogen fixation.

Based on sequence homology, XisA and XisC have been included in integrase family of tyrosine recombinase while XisF shows similarity with resolvase family (Carrasco *et al.*, 1994, Esposito *et al.*, 1997). XisA recognizes 11 base pair target sites CGGAGTAATCC flanking the 11 kb *nifD* element (Lammers *et al.*, 1986). XisC recognizes 16 bp sequence CACAGCAGTTATAATG (Golden *et al.*, 1987). XisA and XisC show 61% similarity and 43% overall identity in *Anabaena* PCC 7120 but they do not complement each other (Carrasco *et al.*, 1995). The tyrosine integrase family has been defined by the conservation of catalytic tetrad R-H-R-Y; however in few cases histidine has been replaced with other amino acids for e.g. XisA and XisC contain tyrosine in place of histidine (Nubes-Duby *et al.*, 1998). Substitution of Tyr to Phe in XisC resulted in the loss site-specific recombination activity (Carrasco *et al.*, 2005). More than 105 proteins have been found to belong to integrase family and the homology tree

analysis revealed that XisA and XisC proteins are distinct from all other members (Nubes-Duby *et al.*, 1998).

Site specific recombinases are multimers and the subunits have been characterized for the catalytic activity and specificity. In Tn3 resolvase, N-terminal is involved in catalysis while the C-terminal contains the sequence specificity (Akopian *et al.*, 2003). Lambda integrase of tyrosine recombinase family has been characterized in to three domains: 1-64 amino acid N-terminal domain, involved in the arm type binding through protein-protein interaction, central domain comprising of 65-169 amino acids involved in core type binding and the C-terminal domain, comprising of residues 170-356, is capable DNA cleavage, strand exchange and ligation steps in the recombination reaction. The carboxyl region also contains the conserved RHRY characteristic of tyrosine recombinase family. C-terminal region of λ integrase was shown to be sufficient for recombination and also involved in the regulation (Kazmierczak *et al.*, 2002). The carboxyl region of XisA and XisC is similar to integrase domain and their N-terminals do not show any sequence similarity with the known integrase family members (Esposito *et al.*, 1997).

In this chapter, the site specific recombination and loss substrate plasmids by C-terminal region of XisA, XisC and chimeric excisase with N-terminal of XisA and C-terminal of XisC in *E. coli* has been studied.

5.2 Materials and methods

5.2.1 Bacterial strains and plasmids used in the study are given in **Table 5.1**

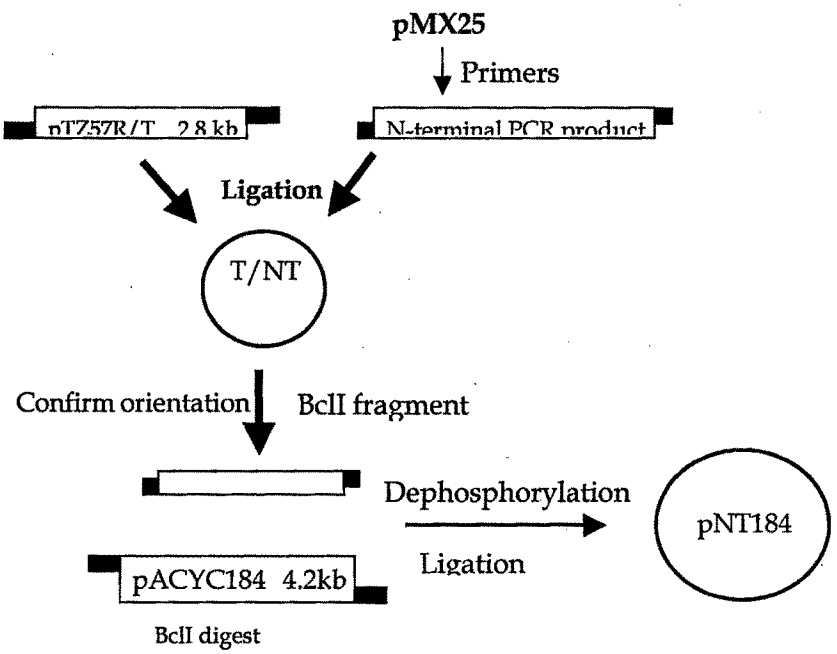
E. coli / Plasmids	Genotype / Features	Source/Reference
Strains		
JM101	*FΔtraD36lacI^{q}Δ (lacZ)M15 proA$^+$B$^+$/supE thi Δ (lac-proAB)*	Bolivar *et al.*, 1977
GN11	JM101 (pNT184 & pMX32)	This study
GN12	JM101 (pCT184 & pMX32)	This study
GN13	JM101 (pNTCT184 & pMX32)	This study
GN14	JM101 (pCT-A-pTTq18 & pAM1500)	This study
GN15	JM101 (pCT-C-pTTq18 & pAM1500)	This study
GN16	JM101 (pCT-C-pTTq18 & pMX32)	This study
GN17	JM101 (pXisR-184-gm & pMX32)	This study
GN18	JM101 (pXisR-184-gm & pAM1500)	This study
GN19	JM101 (pNT-T & pAM1500)	This study
	Plasmids	
pTTQ 18	Amp^R	Stark *et al.*, 1987
pGM160	Gen ^{R}	Muth *et al.*, 1989
pTTQ 18-gm	contains1.6 kb HindIII fragment from pGM160 with gentamycin resistance	This study
pTZ57R	Amp^R	Mead *et al.*,1986
pACYC 184	Chl^R	Chang *et al.*,1978
pMX32	Substrate plasmid of *xisA*, defective in *xisA*, Amp^R,Kan^R,Tet^R	Lammers *et al.*,1986
pNT184	Contains N-terminal of XisA at BclI site, Chl^R	This study
pCT184	Contains C-terminal of XisA at XbaI-BamH1 site, Chl^R	This study
pCTNT 184	Chl^R, Contains both N-terminal and C-	This study

	terminal of XisA	
pCTC	Contains C-terminal of XisC under *tac* promoter, AmpR, Gen^R	This study
pCTA	Contains C-terminal of XisA under tac promoter, Amp^R	This study
pXisR	N-terminal XisA-C-terminal XisC, Gen^R	This study
pAM1500	Substrate plasmid for XisC, Chl^R	Carrasco *et al.*, 2005

5.2.2 Construction of Plasmids

5.2.2.1 Construction pNT184 plasmid containing of N-terminal of XisA:

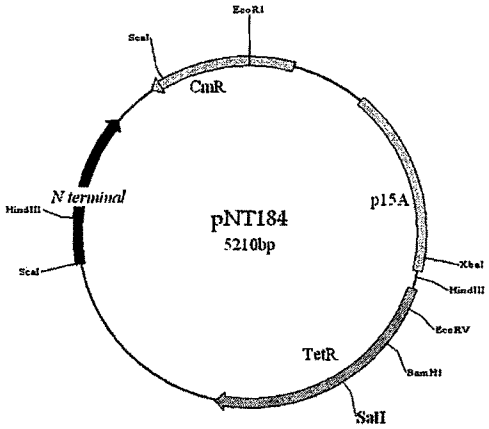
Strategy:

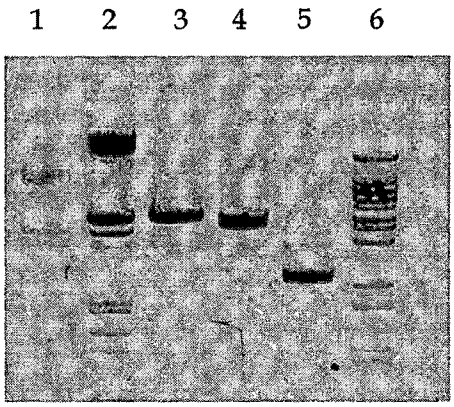


N-terminal of XisA was amplified using F- (5'GGTGATCATAATGTT TGCACTGAGCAGTGT 3') and R (5' GGTGATCATCAGCGTAAACCAAAA ACTGCT 3') with pMX25 as template. Temperature profile - 94°C for 3 min, (94°C/30 s, 42°C/30 s, 72°C 90 s) for 5 cycles and (94°C/30 s, 52°C/30 s, 72°C/ 90 s) for 25 cycles, 72°C -10 minutes final extension. 1 kb N-terminal amplicon

was digested with BclI and ligated with BclI digested pACYC184 to generate pNT184. Size of the plasmid is 5.2 kb.

Figure 5.1 Plasmid map and restriction digestion pattern of pNT184

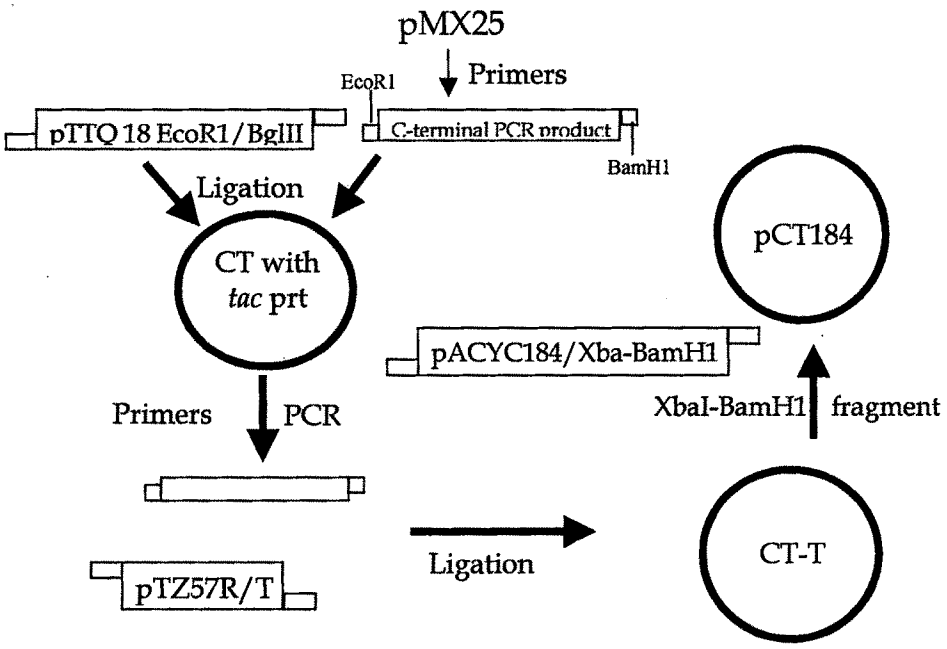




1. Control plasmid
2. Mol. Wt. Marker
3. pNT184/EcoRI digest
4. pNT184/BamHI digest
5. pNT184 HindIII digest
6. Mol Wt. Marker

5.2.2.2 Construction pCT184 plasmid containing C- terminal of XisA

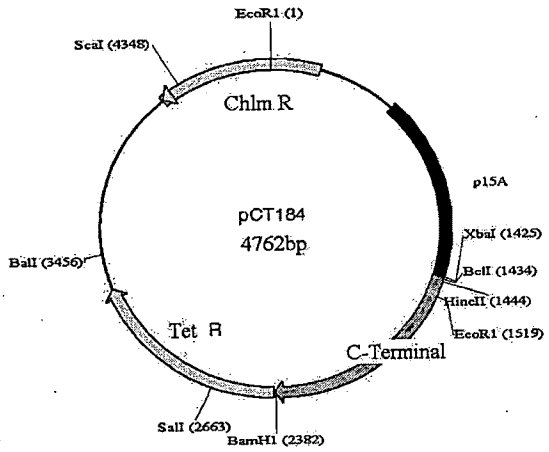
Strategy used:

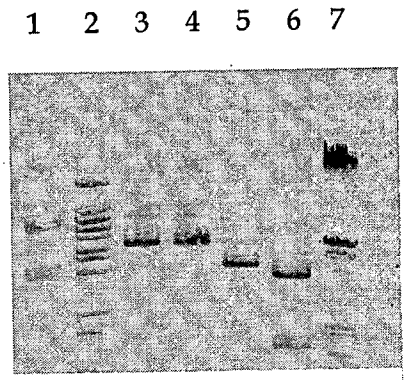


Cloning of C-terminal was done in two steps as CT does not contain promoter. In the first step, CT was amplified using F-5′ TGAG TCGACTGATC AAAGCATTGAGCAGAT 3′ and R-5′ GGGAATTCAGAATGGGCGAGATAT AATGC 3′. Temperature profile- 94°C for 3 mins, (94°C/30 s, 42°C/30 s, 72°C/ 90 s) for 5 cycles and (94°C/30 s, 62°C/30 s, 72°C/90 s) for 25 cycles, 72°C -10 minutes final extension.1 kb C-terminal amplicon were digested with EcoR1/BclI and used for ligation with EcoR1/BamH1 linearized pTTq18 to generate C-terminal under the *taq* promoter, pCT-A.

In second round of PCR primers specific for *tac* promoter 5′ GGTGATCAAGCTGTTGACAAT TAATCATCG 3′ was used as forward primer PCR was carried out to amplify C-terminal under the *tac* promoter. The amplicons were first cloned in pTZ57R vector by blunt end cloning in to EcoRV site. 1 kb XbaI/BamH1 fragment containing C-terminal was sub cloned in pACYC184-gm digested with same enzymes to generate pCT184. Size of pCT184 is 4.7 kb.

Figure 5.2 Plasmid map and restriction digestion pattern of pCT184



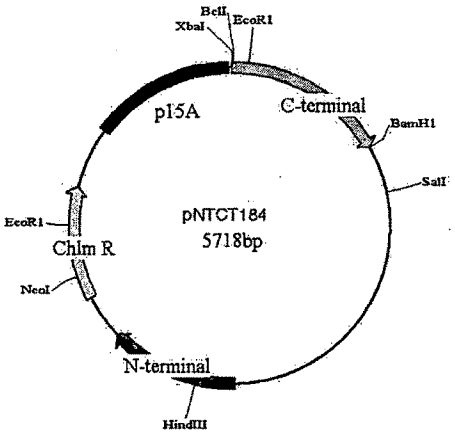


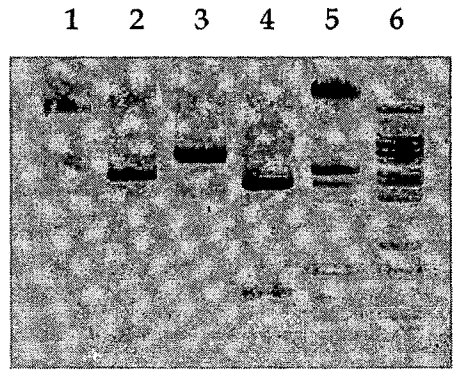
1. Control plasmid
2 & 7. Mol Wt. Marker
pCT184 digested with
3. HindIII 4. BamH1
5. XbaI/BamH1 6. EcoRI

5.2.2.3 Construction pNTCT184 plasmid containing both N-terminal and C-terminal of XisA

1kb XbaI-BamH1 fragment containing C-terminal under *tac* promoter from pCT-T was ligated with NT digested with XbaI-BamH1 to generate pNTCT 184.

Figure 5.3 Plasmid map and restriction digestion pattern of pNTCT184

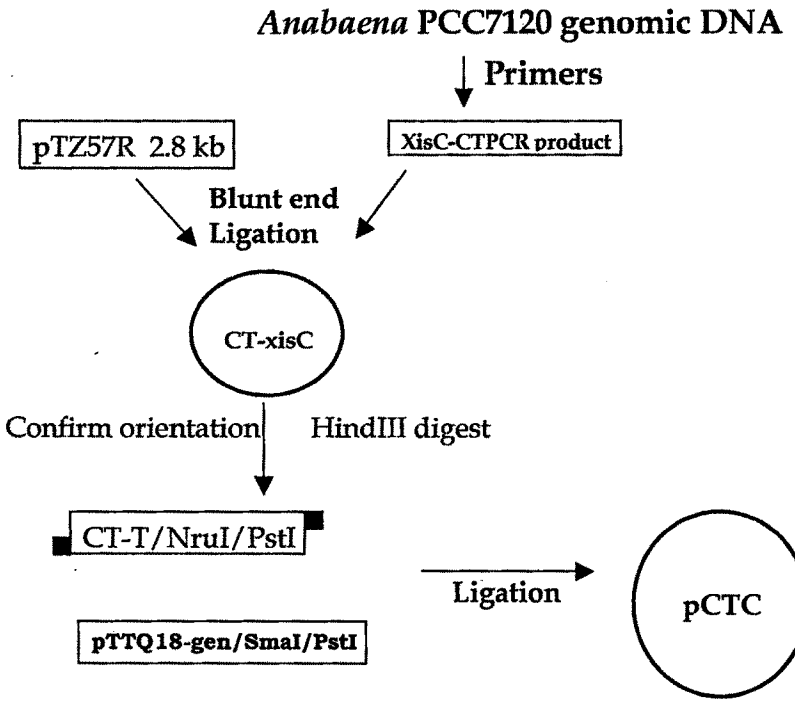


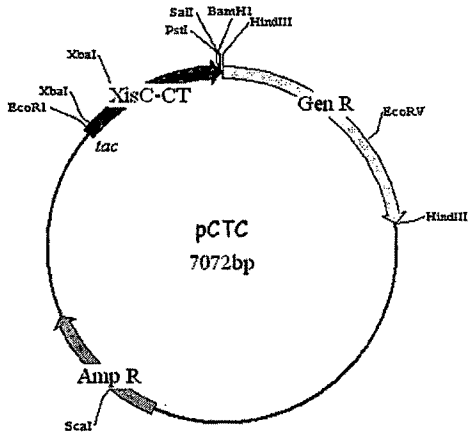


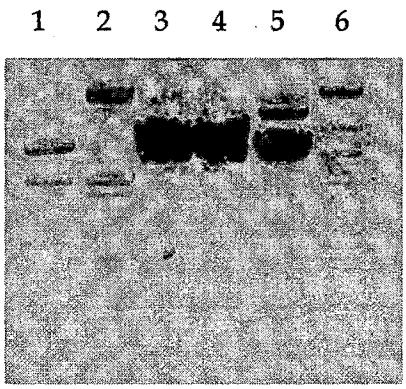
1. Control plasmid
2. pNTCT184/XbaI/BamHI digest
3. pNTCT184/SalI digest
4. pNTCT184/ EcoRI digest
5 & 6. Mol Wt. Marker

5.2.2.4 Construction of pCT-C containing C-terminal of XisC

C-terminal of XisC was amplified using 5′ CCTGGGGCGTAAACCAAA AACT GCGATGTTAAACGTGATAAT 3′ and 5′ GGAACAGGTGCAAGATGC GAGCTAC 3′as reverse primer with *Anabaena* genomic DNA as template. Temperature profile- 94°C for 3 mins, (94°C/30 s, 42°C/30 s, 72°C 90 s) for 5 cycles and (94°C/30 s, 52°C/30 s, 72°C 90 s) for 25 cycles, 72°C -10 minutes final extension. 850 base pair PCR product was first cloned into pTZ57R vector by blut end cloning at EcoRV site. 0.9 Kb NruI/PstI fragment containing C-terminal of XisC was sub cloned into pTTq18-gm at SmaI/PstI site to generate C-terminal of XisC under *tac* promoter.



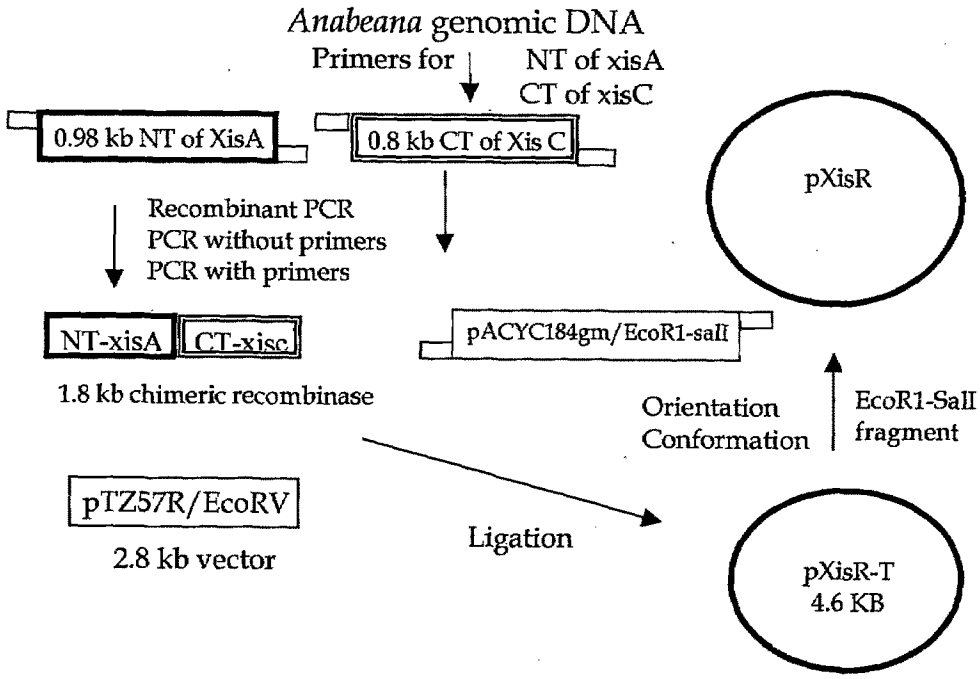




1. Control plasmid
2 & 6. Mol. Wt. Marker
3. pCTC/ EcoRI digest
4. pCTC/BamHI digest
5. pCTC/HindIII digest

Figure 5.4 Plasmid map and restriction digestion pattern of pCTC

5.2.2.5 Construction of chimeric excisase

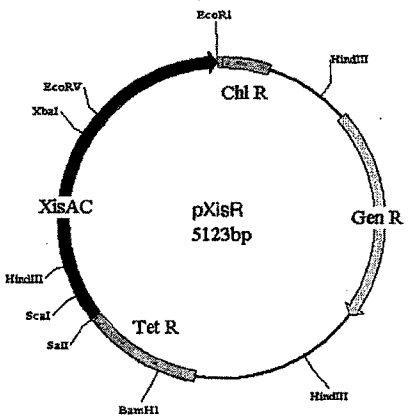


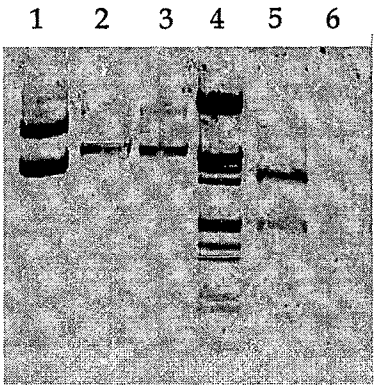
In case of chimeric excisase N-terminal of XisA and C-terminal of XisC were amplified separately and by recombinant PCR, chimeric excisase containing N-terminal of XisA and C-terminal of XisC was generated.

N-terminal of XisA was amplified by using forward primer 5′ GGTGATCATAATGTTTGCACTGAGCAGTGT 3′ and reverse primer 5′ ATTATCACG TTTAACATCGCAGTTTTTGGTTTACGCCCCAGG 3′. C-terminal of XisC was amplified as described above. Both the amplified products were purified used as template for recombinant PCR reaction with forward primer of N-terminal of XisA and reverser primer of XisC. In the first round of PCR, no primers were added, after the completion of 25 cycles, primers were added, and the reaction was continued for another 25 cycles to generate recombinant PCR. Cycle 1: (without primers) 94°C for 3 mins, (94°C/30 s, 42°C/30 s, 72°C 90 s) for 5 cycles and (94°C/30 s, 50°C/30 s, 72°C 90 s) for 25 cycles, 72°C -10 minutes final. Cycle 2: (using 5 µl of PCR product from first reaction as template and with primers). 94°C for 3 mins, (94°C/30 s, 42°C/30 s,

72°C 90 s) for 5 cycles and (94°C/30 s, 50°C/30 s, 72°C 90 s) for 25 cycles, 72°C - 10 minutes final

Size of N-terminal of XisA is 850 bp, while the C-terminal of XisC is of 900 bp. The recombinant excisase is of 1.75 kb, which was purified and was first cloned in to pTZ57R vector. From this vector, 1.75 kb EcoR1/SalI fragment was cloned in pACYC184 vectors at the same sites to generate pXisR.





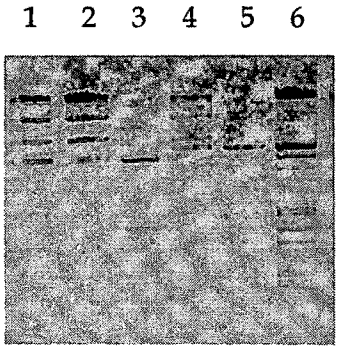
1. Control plasmid
2. pXisR/EcoRI digest
3. pXisR/BamHI digest
4. Mol.Wt. Marker
5. pXisR/EcoRI/SalI digest
6. XisR PCR product

Figure 5.5 Plasmid map and restriction digestion pattern of pXisR

5.2.3 Generation of double transformants

In order to study the domain function, double transformants as described in table 5.1 were generated selected, and confirmed by restriction digestion profile of isolated plasmids (Figure 5.6-5.13).

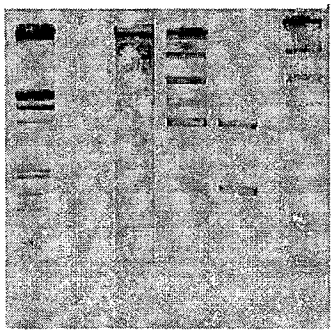
Figure 5.6 Restriction digestion profile of NT184 and pNTCT184 with pMX32



Lane 1 pMX32 + pNT184/EcoR1 digest
Lane 2 pMX32 EcoR1 digest
Lane 3 pNT184 EcoR1 digest
Lane 4 pMX32 + pNTCT184/EcoR1 digest
Lane 5 pNTCT184/EcoR1 digest
Lane 6 Mol. Wt. Marker λ /EcoR1/HindIII digest

Figure 5.7 Restriction digestion profile of pCT184 with pMX32

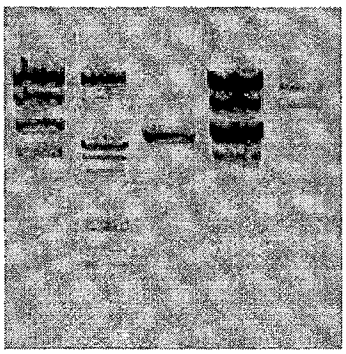
1 2 3 4 5



1. Mol. Wt. Marker λ/EcoR1/HindIII digest
2. Control plasmid
3. pCT184 + pMX32 digested with EcoR1
4. pCT184 digested with EcoR1
5. pMX32 digested with EcoR1

Figure 5.8 Restriction digestion profile of pCTC with pMX32

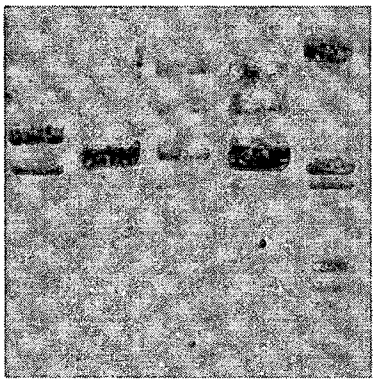
1 2 3 4 5



1. pMX32 digested with EcoR1
2. Mol. Wt. Marker λ/EcoR1/HindIII digest
3. pCTC digested with EcoR1
4. pCT184 + pMX32 digested with EcoR1
5. Control plasmid

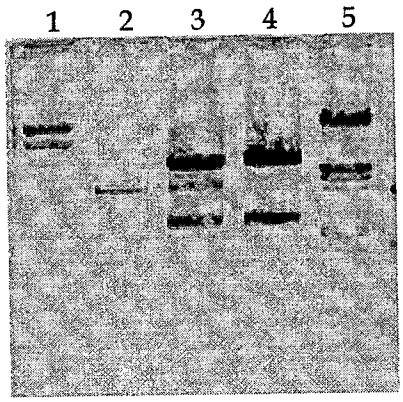
Figure 5.9 Restriction digestion profile of pXisR with pMX32

1 2 3 4 5



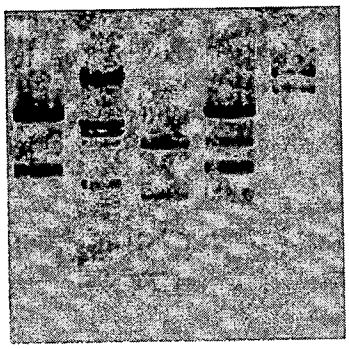
1. Control plasmid
2. pXisR/EcoRI digest
3. pMX32/EcoRI digest
4. pXisR + pMX32/EcoRI digest
5. Mol. Wt. Marker

Figure 5.10 Restriction digestion profile of pNT-T with pAM1500



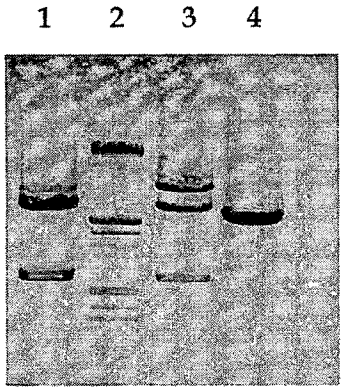
1. Control plasmid
2. pNT-T digested with EcoR1
3. pNT-T + pAM1500 digested with EcoR1
4. pAM1500 digested with EcoR1
5. Mol. Wt. Marker λ/EcoR1/HindIII digest

Figure 5.11 Restriction digestion profile of pCTC and pAM1500



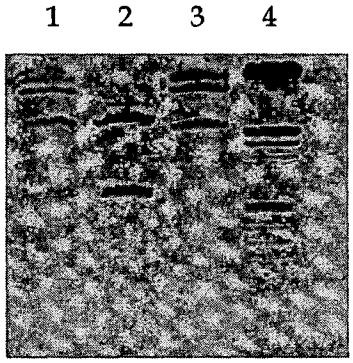
1. pAM1500 digested with EcoR1
2. Mol. Wt. Marker λ/EcoR1/HindIII digest
3. pCTC digested with EcoR1
4. pCTC + pAM1500 digested with EcoR1
5. Control plasmid

Figure 5.12 Restriction digestion profile of pCT-A and pAM1500



1. pAM1500 digested with EcoR1
2. Mol. Wt. Marker
   λ/EcoR1/HindIII digest
3. pCTA + pAM1500 digested
   with EcoR1
4. pCTA digested with EcoR1

Figure 5.13 Restriction digestion profile of pXisR and pAM1500



1. pXisR + pAM1500 digested
   with EcoR1
2. pAM1500 digested with EcoR1
3. pXisR digested with EcoR1
4. Mol. Wt. Marker
   λ/EcoR1/HindIII digest

5.2.4 Monitoring the rearrangement in various *E. coli* strains

A single *E. coli* colony containing substrate plasmid pMX32/pAM1500 and Region containing plasmid i.e.CT of XisA/NT of XisA/CT of XisC/ Chimeric Excisase was grown overnight in 10ml Luria Broth respective antibiotics. Antibiotic concentration used was Ampicillin (100μg/ml), Kanamycin (50μg/ml), Chloramphenicol (40μg/ml), Gentamycin (40μg/ml). In the experiments involving *tac* promoter containing plasmid, 0.1mM IPTG was used for induction. 1 ml culture was washed thrice with saline and 1% (100μl) of

cells was inoculated in Luria broth /M9 minimal medium containing respective antibiotics. From the remaining cells 10^{-4} dilution was made using saline and plated on Luria agar with antibiotics and X-gal as 0 h plating to determine the number of white colonies present in the starting culture. Kanamycin was added in case of pMX32 substrate plasmid to ensure that the rearranged colonies were eliminated. The cultures were allowed to grow for 24 h at 37°C. After 24 h, the dilutions were made as above and plated on X-Gal and antibiotic containing plates. The plates were kept at 37°C and the total excision was calculated using this formula.

$$\text{Excision (\%)} = \frac{\text{Total number of white colonies}}{\text{Total number of colonies}} \times 100$$

5.2.6 Monitoring the endonuclease activity of regions of XisA and XisC

For monitoring the endonuclease activity, protocol was similar to described above except 0 hour plating was not done and appropriate aliquot of the dilution of 24h grown culture was plated on Luria agar plate with and without antibiotic.

Endonuclease activity was calculated as:

Endonuclease activity (%) = (Number of colonies on antibiotic plate*/ Number of colonies on LA plate) × 100

*Antibiotic resistance of the substrate plasmid

All data are analyzed statistically using Prizm3 software. All results are presented as Mean ± S.E.M format.

5.3 Results

5.3.1 Excision analysis of XisA N- and C-terminal regions

N-terminal of XisA corresponding to 1-287 amino acid regions along with *xisA* promoter was used for monitoring the excision of the substrate plasmid pMX32 in Luria broth as well as in M9 minimal medium. No excision was found after 24h of growth in both medium (Table 5.2). C-terminal region corresponding to 203-472 amino acid sequence cloned under *tac* promoter in the pTTQ18 plasmid and was used for the excision studies with pMX32 plasmid. In Luria broth after 24 h, it showed ~6% rearrangement while in M9 minimal medium it showed around 71% of the rearrangement. Plasmid isolated from the white colony showed similar restriction digestion pattern corresponding to XisA. When N-terminal and C-terminal was cloned separately on the same plasmid and checked for the rearrangement, the excision frequency was ~ 4% in Luria broth while it was ~ 62% in M9 minimal medium (Table 5.3).

5.3.2 Excision analysis of XisC C-terminal region

Similar to C-terminal of XisA, C-terminal of XisC corresponding to 243-498 amino acids was cloned under *tac* promoter in pTTQ 18 plasmid and used for monitoring the excision frequency with substrate plasmid pAM1500. It didn't show any excision after 24 h in Luria broth as well as in M9 minimal medium (Table 5.4).

To check the specificity of C-terminal region, C-terminal of XisA was used to check the excision of pAM1500, substrate plasmid of XisC and vice versa. Results showed that C-terminal of XisA and XisC do not show cross reactivity as the excision was 0% (Table 5.5-5.6).

5.3.3 Excision analysis of chimeric excisase

Chimeric excisase containing N-terminal (1-287 amino acids) of XisA and C-terminal (243-498 amino acids) of XisC was constructed using recombinant PCR and used for the excision studies with substrate plasmid of XisA and XisC.

It showed around ~ 5% excision frequency with pAM1500, substrate plasmid of XisC and no excision with XisA substrate plasmid, pMX32 (Table 5.7). N-terminal of XisA alone does not bring about excision of pAM1500 (Table 5.8).

5.3.4 Endonuclease activity of excisase regions and chimeric excisase

N- and C-terminal regions of XisA, C-terminal region of XisC and chimeric excisase were checked for the endonuclease activity by specific loss of target site containing plasmid. The N-terminal region of XisA did not show any activity while the C-terminal region showed around 40% loss in the target site containing plasmid, similar to the plasmid containing both N-terminal and C-terminal regions. However, C-terminal of XisC and chimeric excisase did not show any loss of target site containing plasmid (Table 5.9).

Table 5.2 Excision of *nifD* element of *Anabaena* PCC 7120 in *E. coli* in the presence of N-terminal of XisA

Media/Time (h)	Number of Blue colonies	Number of White colonies	Excision (%)
E. coli strain GN11			
LB/24hr	173 ± 16	3.5 ± 1	0
M9/24hr	155 ± 14	2 ± 1	0

Table 5.3 Excision of *nifD* element of *Anabaena* PCC 7120 in *E. coli* in the presence of C-terminal of XisA and C-terminal along with N-terminal

Media/Time (h)	Number of Blue colonies	Number of White colonies	Excision (%)
E. coli strain GN12			
LB/24hr	348 ± 24	23 ± 3	6.3 ± 0.6
M9/24hr	68 ± 8	176 ± 20	71 ± 4.5
E. coli strain GN13			
LB/24hr	367 ± 20	16 ± 1.5	4.1 ± 0.3
M9/24hr	91 ± 24	146 ± 16	62 ± 6

Table 5.4 Excision frequency of C-terminal of XisC with pAM1500 plasmid

Media/Time (h)	Number of Blue colonies	Number of White colonies	Excision (%)
E. coli strain GN15			
LB/24hr	423 ± 18	2 ± 1.4	0
M9/24hr	301 ± 14	3 ± 2	0

Table 5.5 Excision frequency of C-terminal of XisA with pAM1500 plasmid

Media/Time (h)	Number of Blue colonies	Number of White colonies	Excision (%)
E. coli strain GN14			
LB/24hr	312 ± 11	3 ± 1.5	0
M9/24hr	244 ± 16	2 ± 1	0

Table 5.6 Excision frequency of C-terminal of XisC with pMX32 plasmid

Media/Time (h)	Number of Blue colonies	Number of White colonies	Excision (%)
E. coli strain GN16			
LB/24hr	210 ± 38	4 ± 2	0
M9/24hr	258 ± 28	6 ± 4	0

Table 5.7 Excision frequency of Chimeric recombinase XisR with pAM1500 and pMX32

Media/Time (h)	Number of Blue colonies	Number of White colonies	**Excision (%)**
E. coli strain GN17			
LB/24hr	388 ± 24	4 ± 3	0
M9/24hr	268 ± 28	2 ± 2	0
E. coli strain GN18			
LB/24hr	600 ± 28	34 ± 3.4	5.1 ± 0.5
M9/24hr	301 ± 14	17 ± 2	5.1 ± 0.43

Table 5.8 Excision frequency of N-terminal of XisA with pAM1500

Media/Time (h)	Number of Blue colonies	Number of White colonies	Excision (%)
E. coli strain GN16			
LB/24hr	189 ± 22	2 ± 0.6	0
M9/24hr	159 ± 18	1 ± 0.4	0

Table 5.9 Endonuclease activitity of C-terminal of XisA and C-terminal of XisC and recombinant XisR

Media/Time (h)	Number of colonies on LA	Number of colonies on LA+Amp	Excision (%)
E. coli strain GN15			
LB/24hr	222 ± 24	210 ± 32	0
M9/24hr	211 ± 21	234 ± 30	0
E. coli strain GN12			
LB/24hr	380 ± 78	145 ± 59	36 ± 5.5
M9/24hr	1325 ± 78	796 ± 72	40 ± 3.3
E. coli strain GN18			
LB/24hr	359 ± 53	345 ± 59	0
M9/24hr	325 ± 44	306 ± 63	0

5.4 Discussion

XisA and XisC belong to integrase family of tyrosine recombinase. Results reported here show that the C-terminal region of XisA corresponding to 203-472 amino acids is sufficient to bring about the excision of *nifD* element comparable to that of complete XisA protein. Extent of the excision was not enhanced by presence of its N-terminal and N-terminal region alone had no excision activity. This implies that the *Int* domain of C-terminal could be responsible and sufficient for the excision. C-terminal region of XisA does not bring about excision with substrate plasmid of XisC suggesting that the specificity regions are not present in the N terminal. N-terminal region of XisA does not appear to play any role as the excision frequency and the specificity of the C-terminal region of XisA was similar to XisA. Similar role for carboxyl terminal has been

reported in case of λ integrase where C-terminal region alone has showed topoisomerase activity ie capable of cutting, strand exchange and ligation, with reduced specificity (Tirumalai *et al.*, 1997), which was enhanced by the presence of N-terminal involved in arm type binding (Jessop *et al.*, 2000).

XisA and XisC show high similarity in amino acid sequences including conserved residues of *Int* domain (Carrasco *et al,*. 1995). In contrast to C-terminal region of XisA, XisC C-terminal region did not show excision of its substrate plasmid, suggesting the requirement of N-terminal for its activity. This view is supported by the chimeric excisase containing N-terminal of XisA and C-terminal of XisC was able to cleave the substrate plasmid of XisC at around 5% frequency which was less than shown by whole *xis*C which shows around 10% (Carrasco *et al,*. 2005). Hence, supplementing the N-terminal of XisA compliments the N-terminal of XisC and shows the excision albeit at a lower level than whole XisC. Hybrid recombinases have been used to understand the catalytic and specificity functions. Hybrid FLP recombinase with N-terminal of FLP recombinase of yeast *Saccharomyces* replaced by R-recombinase from *Zygosaccharomyces rouxii* recognized FLP recombination site, but the hybrid FLP was catalytically inactive *in vivo* (Chen *et al.*, 1991). *Int* domain of RAG1, involved in the VDJ recombination in lymphocytes, when replaced with HinA recombinase *Int* domain, retained the VDJ recombination activity (Spanopoulou *et al.*, 1996). In case of serine recombinases, specificity determining C-terminal region of Tn3 resolvase was fused with DNA recognition domain from the mouse transcription factor Zif268 conferred the specificity of mouse transcription factor (Akopian *et al.*, 2003).

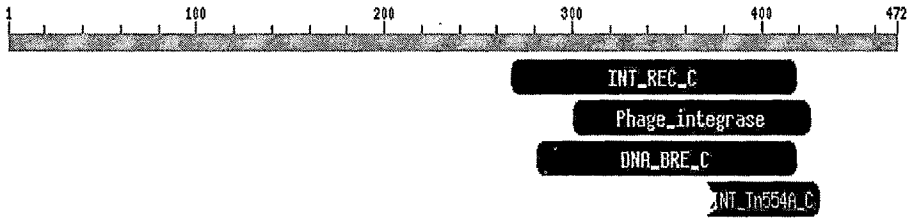
The target site of XisA CGGAGTAATCC contains a 3 base inverted repeats, separated by 4 nucleotides, characteristic feature of integrase target sites. XisA along with the recombinase activity shows the endonuclease activity with specific loss of target site containing plasmid. C-terminal of the XisA showed loss of substrate plasmid indicating the endonuclease activity, which

was comparable with those shown by XisA. In contrast chimeric XisC did not show any loss of substrate plasmid.

This data along with the earlier data suggests that even though XisA and XisC show very good similarity (figure 1), functionally and structurally they seem to be different. Using structural programmes like pDB, structure of XisA is found to be very similar to XerC-XerD recombinase while XisC structure is similar to λ integrase. XisC requires the N-terminal for its activity while XisA does not. XisA possesses the endonuclease activity while the XisC does not possess. N-terminal of XisA is capable of partially complimenting the N-terminal function of XisC. Domain analysis of XisA and XisC, obtained by the conserved domain search utility of BLAST, showed presence of *Int* domain but the extent of homology differs with other integrase family members (figure 5.18). This may account for the difference in their activities. N-terminal XisA/C-terminal XisC chimeric recombinase shows the Xer recombinase like structure, thus the presence of N-terminal of XisA significantly alters the structure of carboxyl terminal of XisC. Even though XisA and Chimeric recombinase show structural similarity, specificity of site-specific recombination and endonuclease activities are different. Further characterization of the recombination mechanism by XisA and XisC requires the purified proteins, which has been the major hindrance factor even though their genes had been cloned for many years ago.

Figure 5.14 Conserved domains of XisA and XisC as determined by conserved domain search utility of BLAST.

(a) XisA conserved domains



(b) XisC conserved domains

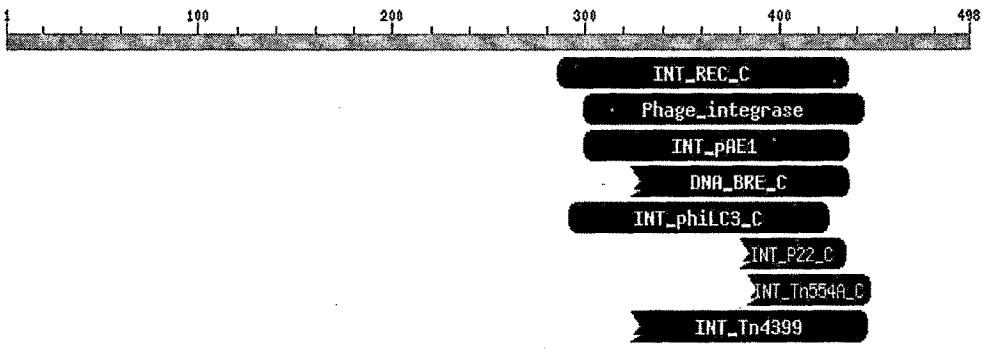


Figure 5.15 Sequence similiarity between XisA and XisC

```
XisA- --MQNQGQ------------------KYQQAFADLEPLSSTDGSFLGSSLQAQQQREHM
XISC_ SGQNQGNCEETYSTSTHEYANADSTDWFADMFADFEPQNTTDGSYRGTMAKIKATELQY
        ****:                    * : : ***:** .:****: *:  : :  . :

XisA RTKVLQDLDKVNLRSKSAKTKVSVRESNGSLQLRATLPIKPRDKDTNGTGRKQYNLSLNI
XisC QAVFEQKLVEANTNLKRERIRVSIKQTGNSLQLRATLPLKPGD-GSLGKTKKQYDLSLGI
     :: . *.* :.* . *  : :**::::..*********:** * .: *. :***:***.*

XisA PANLDGLKTAEEEAYELGKLIARKTFEWNDKYLG-KEATKKDSQTIGDLLEKFAEEYFKT
XisC PANLEGLKTAIEESYELGKLIARHTFEWNEKYLGIKSREKQEIKTIGELLDKFEEKYYQT
     ****:***** **:*********:*****:**** *.  *:: :***:**:** *:*::*

XisA HKRTTKNKHTFFYYFSRTQRYTNSKDLATAEN---LINSIEQIDKEWARYNAARAISAFC
XisC RQKTITSQNTFPNYISVIKRNFPLTHLATKENFEEIINSVQGNKKN----ELIAVTSVFI
     :::* ..::**  *:*  :*    ..*** **   :***::  .*:    :   . *.*

XisA ITFNIEIDLSQYSKMPDRNSRNIPTDAEILSGITKFEDYLVTRGNQVNEDVKDSWQLWRW
XisC KTFNLGFQLDVKRDNVTPAHREIPEDDKIIYSFDLFEKFALNRKNTNISDEIDTWEMWRW
      ***: ::*.   .      *:** * :*: .:  **.: :.* *   .*  *:*::***

XisA TYGMLAVFGLRPREIFINPNIDWWLSKENIDLTWKVDKECKTGERQALPLHKEWIDEFDL
XisC VYGMLATFGLRPRELFVQPDINWWMSPQNLDHTWKVNKNTKTGYREVIPFVPEWIELFDL
     .*****.*******:*::*:*:**:* :*:* ****:*: *** *:.:*:  ***: ***

XisA RNPKYLEMLATAISKKDKTNHAEITALTQRISWWFRKVELDFKPYDLRHAWAIRAHILGI
Xisc KNPKPLKILEKKVTKIASVQN--INWMRRDISRWFRKVGIEFQPYDLRHACAIRAHLQGI
     :*** *::* . ::*  ..::  *. : : ** ***** ::*:******* *****: **

XisA PIKAAADNLGHSMQVHTQTYQRWFSLDMRKLAINQALTKRNEFEVIREENAKLQIENERL
XisC PIKAAADNLGHTVDEHTKTYQRWFGIENRKKAFGEVISQKSLIELQKNEILALRMENERL
     ***********::: **:******.:: ** *:.:.:::::. :*: ::*   *::*****

XisA RMEIEKLKMEIAYKNS---------
XisC RLEVEKLKFSTTKNPEDCEQLYHQG
     *:*:****:. : : .
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