

**Regulation and functional analysis of a geminiviral  
DNA  $\beta$   
satellite encoded gene**

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## Abstract

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Geminiviruses (family *Geminiviridae*) are characterized structurally by twinned (geminata) morphology of virions (ca. 18-30 nm) and genetically by a genome comprising one or two small circular single stranded DNA (ssDNA) molecules and they are responsible for major crop losses worldwide. The genus *Begomovirus* (type member *Bean golden yellow mosaic virus*) is the largest genus of the family *Geminiviridae*. The members of this genus have either monopartite or bipartite genomes. They are transmitted by whiteflies and infect only dicotyledonous plants.

DNA  $\beta$  molecules are symptom modulating single-stranded sat-DNA molecules which are associated with certain monopartite begomoviruses. These molecules are around half the size (approximately 1350 nt in length) of their helper viruses and rely on the helper begomovirus for movement in plant tissues, replication and plant-to-plant transmission by the whitefly (*Bemisia tabaci*). They contribute to production of symptoms and enhance helper virus accumulation in certain hosts. DNA  $\beta$  molecules encode a single gene, called  $\beta C1$ , on the complementary strand which is important for pathogenicity and suppression of post transcriptional gene silencing. In this study the regulation of  $\beta C1$  gene expression, a host factor interacting with  $\beta C1$  and its role in the pathogenicity of DNA  $\beta$  are described.

Transient expression studies using *Nicotiana tabacum* plants and GUS as a reporter gene, identified the sequences important for transcription of  $\beta C1$  from DNA  $\beta$  associated with *Cotton leaf curl Multan virus* (CLCuMV). A 68 nt fragment (between -139 to -207), which contains a G-box motif was sufficient for DNA  $\beta$  promoter activity. Deletion of this region also led to loss of DNA  $\beta$  replication capacity. Mutation of the G-box, located at 143 nucleotides upstream of the  $\beta C1$  start codon, resulted in a two to three times reduction in the DNA  $\beta$  promoter activity. This motif was shown to bind specifically to the nuclear factors isolated from tobacco leaf tissues. Histochemical staining of transgenic tobacco plants expressing the *gus* gene driven by full length DNA  $\beta$  promoter showed phloem

specific localisation patterns. It was concluded that a G-box motif is required for binding of host nuclear factors and is necessary for efficient expression of this phloem specific  $\beta C1$  gene.

An ubiquitin-conjugating enzyme, called SIUBC, was retrieved from screening of a tomato cDNA library, using  $\beta C1$  encoded by DNA  $\beta$  associated with CLCuMV as the bait. The *SIUBC* was shown to complement yeast deficient in the ubiquitin-conjugating enzyme. It is thought that this enzyme is a key factor in the ubiquitin proteasome pathway, which plays a central role in many eukaryotic cellular processes. The authenticity and specificity of this interaction was confirmed both *in vivo*, using a bimolecular fluorescence complementation assay, and *in vitro*. Domain mapping of  $\beta C1$  showed that a myristoylation-like motif is required for the interaction with *SIUBC* in the yeast system and induction of DNA  $\beta$  specific symptoms in host plants.

Western blot analysis showed that expression of  $\beta C1$  in transgenic tobacco plants decreased the level of poly-ubiquitinated proteins as compared with wild type plants. However, the level of expression of homologous *SIUBC* remained stable in these transgenic plants. These results indicated that interaction of  $\beta C1$  with the SIUBC is required for DNA  $\beta$  specific symptom induction possibly through down-regulation of the host ubiquitin proteasome pathway.

Using GFP transgenic *N. benthamiana* plants, the  $\beta C1$  encoded by DNA  $\beta$  associated with CLCuMV showed suppression of post transcriptional gene silencing. This protein inhibited both local and systemic silencing. However, the low level of GFP fluorescence and also the results of RNA analysis in patch co-infiltration assay indicated that  $\beta C1$  is a weak suppressor of local RNA silencing as compared with P19 protein from *Tomato bushy stunt virus*.

A three-way grafting assay and separate patch infiltration assays showed that  $\beta C1$  interferes with the activity of GFP silencing signal. Mutation of Gly103 in  $\beta C1$  which was shown to be required for interaction with SIUBC and induction of DNA  $\beta$  specific symptoms in host plants, had no effect on the silencing suppression activity of  $\beta C1$  protein.

This work has provided a new insight into the importance of a G-box motif in expression of *βCI* gene of DNA  $\beta$  and also for binding to the host nuclear proteins. In addition, interaction with a host factor, *SIUBC*, has been shown to be required for induction of DNA  $\beta$  specific symptoms in experimental plants using ToLCV as a helper virus. However, this interaction was not required for silencing suppression activity of *βCI*. The results of this study can be adapted to determine the mode of pathogenesis and regulation of expression of *βCI* in cotton leaf curl disease.

## **Declaration**

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This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Omid Eini  
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*my beloved wife, Zahra and my precious mother, Zarafshan*

## Abbreviations

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ACMV	<i>African cassava mosaic virus</i>
AD	activation domain
ADK	adenosine kinase
AGO1	ARGONAUTE1
Ala	alanine
ATP	adenosine triphosphate
AYVV	<i>Ageratum yellow leaf curl virus</i>
BCTV	<i>Beet curly top virus</i>
BiFC	bimolecular fluorescence complementation
BLAST	basic local alignment search tool
bp	base pairs
BYMV	<i>Bhendi yellow vein mosaic virus</i>
CaMV	<i>Cauliflower mosaic virus</i>
CBP	calmodulin-binding peptide
cDNA	complementary DNA
CLCuD	cotton leaf curl disease
CLCuMV	<i>Cotton leaf curl Multan virus</i>
CM	common motif
CP	coat protein
DCL	Dicer-like
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dsDNA	double-stranded DNA
dsRNA	dsDNA
E1	ubiquitin activating enzyme
E2	ubiquitin-conjugating enzyme

E3	ubiquitin ligase
EACMV	<i>East African Cassava mosaic Cameroon virus</i>
EDTA	ethylenediamine- <i>tetra</i> -acetic acid
EMSA	electrophoretic mobility shift assay
g	gram(s)
GFP	green fluorescent protein
Gly	glycine
Glu	Glutamine acid
h	hour(s)
HRP	horseradish peroxidase
IR	intergenic region
IPTG	<i>Iso</i> -propyl- $\beta$ -D-thiogalactopyranoside
kb	kilobase pairs
L	litre(s)
LB	Luria broth
M	molar
min	minute(s)
miRNA	microRNA
MOPS	3-N-Morpholinopropanesulfonic acid
MP	movement protein
mRNA	messenger RNA
MSV	<i>Maize streak virus</i>
Ni-NTA	nickel nitrilotriacetic acid
NLS	nuclear localisation signal
NSP	nuclear shuttle protein
nt	nucleotide
OD	optical density
OLCD	okra leaf curl disease
ORF	open reading frame
<i>ori</i>	origin of replication
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction

pRBR	plant retinoblastoma-related protein
PTGS	post-transcriptional gene silencing
PVDF	polyvinylidene fluoride
RACE	rapid amplification of cDNA ends
RdDM	RNA-directed DNA methylation
RdRP	RNA dependent RNA polymerase
REn	replication-enhancer protein (encoded by <i>C3</i> or <i>REn</i> )
Rep	replication-associated protein
RF	replicative form
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
RNAi	RNA interference
RT-PCR	reverse transcription-PCR
s	Second(s)
Sat-DNA	satellite DNA
SCR	satellite conserved region
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SGS	Suppressor of gene silencing
siRNA	small interfering RNA
S-PTGS	sense post transcriptional gene silencing
SqLCV	<i>Squash leaf curl virus</i>
SSC	standard sodium citrate
ssDNA	single-stranded DNA
SS-DNA	salmon sperm DNA
ssRNA	single-stranded RNA
TBE	tris-borate-EDTA
TGMV	<i>Tomato golden mosaic virus</i>
TGS	transcriptional gene silencing
ToLCV	<i>Tomato leaf curl virus</i> (Australian isolate)
TBSV	<i>Tomato bushy stunt virus</i>
TrAP	transcriptional activator protein

Tris	tris (hydroxymethyl) aminomethane
TYLCV	<i>Tomato yellow leaf curl virus</i>
TYLCCNV	<i>Tomato yellow leaf curl China virus</i>
UBC	ubiquitin conjugating enzyme
V	volt(s)
YFP	yellow fluorescent protein

## Publications

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Sections of this thesis have been prepared for publication or presentation as follows.

**Omid Eini**; S.A. Akbar Behjatnia; Satish Dogra; Ian B. Dry; John W. Randles and M. Ali Rezaian. Identification of sequence elements regulating promoter activity and replication of a monopartite begomovirus-associated DNA  $\beta$  satellite (accepted in the Journal of General virology).

**Omid Eini**; Satish Dogra; Luke A. Selth, Ian B. Dry; John W. Randles and M. Ali Rezaian. A myristoylation-like motif of  $\beta$ C1 protein encoded by a geminiviral DNA  $\beta$  satellite is required for its pathogenicity and interaction with a tomato ubiquitin-conjugating enzyme (in preparation).

**O. E. Gandomani**, S. Dogra, S.A. Behjatnia, I.B. Dry, J.W. Randles, M.A. Rezaian. (2007). Identification of the minimal sequences required for a Geminivirus satellite  $\beta$ C1 promoter. The 16<sup>th</sup> biennial Australian plant pathology society conference. Adelaide. Page 172

Dongmei Li, S. A. Akbar Behjatnia, Ian B. Dry, John W. Randles, **Omid Eini** and M. Ali Rezaian. (2007). Genomic regions of tomato leaf curl virus DNA satellite required for replication and for satellite-mediated delivery of heterologous DNAs. J. Gen. Virol. 88. 2073-2077

**Eini, G. O.**, Selth L., Dogra S., Randles J., Rezaian A. (2006). A tomato ubiquitin conjugative enzyme interacts with a geminivirus sat-DNA  $\beta$  encoded protein. The 8<sup>th</sup> international congress of plant molecular biology. Adelaide. Page 176

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