Novel interactions of cardamom mosaic virus VPg with cardamom histones H3 and H4

Sankara Naynar Palani¹ · Ramamoorthy Sankaranarayanan¹ · Jebasingh Tennyson¹

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Abstract

The host genome targeting potyviral proteins is sparsely reported. Viral genome-linked protein (VPg) is a multifaceted protein known for its interactions with a suite of host proteins, guides essential viral life cycle processes such as genome replication, translation, genome packing, and antiviral defence. Besides, VPg also plays a crucial role in assisting the transport of nuclear inclusion a protease (NIa protease) into the host nucleus. Apart from that, the role of VPg in the nucleus of the cognate host is not clear. Although NIa protease has been reported for DNase activity, the molecular mechanisms underlying host genome accessibility are not yet understood completely. Here, we employed yeast two hybrid assays to test the cardamom histones H3 and H4 interaction with the VPg and NIa protease of macluravirus *cardamom mosaic virus* (CdMV). Although CdMV NIa protease has the putative histone-binding ER motif of MYST histone acetyltransferase, it did not interact with host histones H3 and H4. Surprisingly, CdMV VPg displayed strong interaction with histone proteins H3 and H4. Leucine prototrophy and β -galactosidase assays were performed which validated VPg interaction with histones. To the best of our knowledge, this study is the first report for the multipartnered potyvirid protein VPg interaction with host histones H3 and H4.

Keywords VPg · NIa protease · Cardamom mosaic virus · ER motif · Histones

Introduction

Cardamom mosaic virus (CdMV), a species of *Macluravirus* genus, belongs to the family *Potyviridae* (Jacob and Usha 2001). Macluraviruses codes for a long polypeptide which is majorly cleaved by nuclear inclusion a protease (NIa protease) (Palani et al. 2018). So far, a plethora of molecular studies has reported the protease activity of NIa protease (Garcia et al. 1992; Dougherty and Semler 1993, Joseph and Savithri 2000; Ray and Hema 2016). However, its role in the host nucleus is still unexplored. The DNase activity of *Pepper vein banding virus* (PVBV) NIa protease has been believed to degrade the host genome to suppress the host defence (Anindya and Savithri 2004; Revers and Garcia 2015). Since the eukaryotic genome is packed as nucleosomes, the action of NIa protease may commence with chromatin remodelling. Interestingly, sequence comparison

showed that essential amino acids of histone-binding ER (Esa1-Rpd3) motif of MYST family are found in CdMV and a few other macluravirus NIa proteases (Fig. 1a). MYST family of histone acetyltransferases (HAT) preferably binds to histones of H3 and H4 (Adachi et al. 2002). The role of this putative ER motif in CdMV NIa protease is yet to be assessed. Moreover, NIa protease depends on the nuclear localization signal (NLS) of viral genome-linked protein (VPg) to enter the host nucleus (Rajamaki and Valkonen 2009). The latent cleavage site of VPg–NIa protease helps the durability of the fusion (Adams et al. 2005). However, the list of host interacting proteins is variable for VPg and NIa protease (Martinez et al. 2016). Particularly, potyvirid VPg is being reported with the escalating number of host–protein interactions to interfere with host physiological pathways to enhance the virus infectivity (Dunoyer et al. 2004; Beauchemin and Laliberte 2007; Huang et al. 2010; Zhu et al. 2014; Rajamaki et al. 2014, Machado et al. 2017).

Reports on virus–host protein interactions provide insights into virus interference in host pathways during the infection. In potyviridae family, VPg and NIa protease are intriguing factors that target the host genome for antiviral defence (Dunoyer et al. 2004; Anindya and Savithri 2004;

✉ Jebasingh Tennyson
jebasinghs@gmail.com

¹ Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu 625021, India

a	Esa1	CTLRHPPGNEI--YRDDYVSFFEIDGRKQRTWCRNLCLLSKLFLLDHKTLYYDVPFLFYC	118
	ArLV	-VDTRLADNMVNLDVGNMIGLVSVKG-----GNANCLLYKDWTMPAH-----V	43
	Wuplv1	-IATHLQTSNVNLDISKMIGLVTVDG-----GQICCIYKDFIIMPAH-----V	43
	BDVA	-IATHLQTSQVNLDAKMIGLVTVDG-----GLICCIYKDFIIMPAH-----V	43
	CdMV	-IDTRLNNNTNLEIMNMIGHVQIGE-----GRLLCLLYKDFIIMPAH-----I	43
	CYNMV	-MATRLPENKLNQVADMIGKVSMSSE-----GTIHCILYKDFIIMPAH-----A	43
		: * : : * : :

Gong et al. 2020). In this study, VPg and NIa protease of CdMV are used to identify the interaction with *Elettaria Cardamomum* histones Histone 3 (H3) and Histone 4 (H4) by employing yeast two hybrid systems.

Materials and methods

Plasmid construction

Elettaria Cardamomum histones H3 (411nt, GenBank accession no. KM591913), H4 (312nt, GenBank accession no. KM591914), CdMV NIa protease, and VPg (642 and 534 nt, GenBank accession no. AJ550378) coding genes were

obtained through primer-based PCR. All forward primers have *EcoRI* site and reverse primers have *XhoI* site for cloning purposes. All of them were amplified using *Taq* DNA polymerase (Sigma), and the PCR products were inserted into *XcmI* digested pXcmKn12 vector using T4 DNA ligase (Thermo scientific). The resulting plasmids were confirmed by DNA sequencing. To construct plasmids for yeast two hybrid interaction assays, H3, H4, NIa protease, and VPg coding sequences were released from the respective pXcm clones by *EcoRI* and *XhoI* digestions. The released fragments were cloned at *EcoRI* and *XhoI* sites, in-frame to LexA DNA-binding domain of pEG202 (bait) and the B42 activation domain of pJG4-5 (prey) yeast two hybrid vectors. The primers used in this study are listed in Table 1.

Fig. 1 a Sequence alignment of macluraviruses N1a protease (ArLV—artichoke latent virus; BDVA—broad-leaved dock virus A, CdMV—cardamom mosaic virus, CYNMV—Chinese yam necrotic mosaic virus, and WuPlv1—Wuhan poty like virus1) with Esa1 (Histone acetyltransferase). Histone-binding residues similar to ER motif are boxed. **b** Growth of bait–prey transformed EGY48 cells on leucine lack minimal media to evaluate CdMV N1a protease–cardamom histones (H4 and H3) interactions. Induced cells were serially diluted and a 10 µl sample was spotted on SD + Gal + Raf–L–W–H–U plates for each dilution. Positive controls pJGH4–pSH17-4 and pJGH3–pSH17-4 transformants were grown up to 10^{-4} dilution. Negative controls pJGH4–pRFHM1, pJGH4–pEG202, pJGH3–pRFHM1, and pJGH3–pEG202 transformants were not grown on the selective medium. The absence of growth in pEGN1a–pJGH4 and pEGN1a–pJGH3 transformants indicated that N1a protease is not interacting with histones H4 and H3. **c** Growth of bait–prey transformed EGY48 cells on leucine lack minimal media to evaluate the interaction of CdMV VPg and cardamom histones (H4 and H3) interactions. Induced EGY48 transformants were serially diluted and 10 µl sample was spotted on SD + Gal + Raf–L–W–H–U plates for each dilution. Positive controls pJGH4–pSH17-4 and pJGH3–pSH17-4 transformants were grown up to 10^{-4} dilution. Negative controls pJGH4–pRFHM1, pJGH4–pEG202, pJGH3–pRFHM1, and pJGH3–pEG202 transformants were not grown on the selective medium. The test pEGVPg–pJGH4 and pEGVPg–pJGH3 transformants were grown up to 10^{-4} dilution. This cell viability assay shows that VPg strongly interacts with histones H4 and H3. **d** Qualitative assessment of the interaction between VPg and H4. Positive control pJGH4–pSH17-4 (1), test pEGVPg–pJGH4 (2), negative controls pJGH4–pRFHM1 (3), and pJGH4–pEG202 (4) transformants were streaked on SD + Gal + Raf + X-gal + L–W–H–U selection medium. VPg–H4 protein interaction induces β -galactosidase expression and produces blue colour on the selection medium. VPg–H4 interaction confirmed by blue colour production similar to the positive control. Negative controls are unable to produce a blue colour. **e** Qualitative assessment of CdMV VPg–cardamom histone H3 interaction. Positive control pJGH3–pSH17-4 (1), test pEGVPg–pJGH3 (2), negative controls pJGH3–pRFHM1 (3), and pJGH3–pEG202 (4) transformants were streaked on SD + Gal + Raf + X-gal + L–W–H–U selection medium. VPg–H3 interaction induces β -galactosidase expression and produces blue colour on the selection medium similar to the positive control. Negative controls are unable to produce a blue colour

Yeast transformation

To perform the interaction assay, the bait and prey constructs were co-transformed into *Saccharomyces cerevisiae* strain EGY48 + (genotype) (Erica Golemis Yeast). It harbours pSH18-34 plasmid for *LacZ* reporter activity (Golemis et al. 2001). A combination of various bait–prey plasmids was transformed to EGY48 cells by lithium acetate method as described previously (Golemis et al. 2001) and plated on synthetic dropout medium (SD) with leucine (L) but without tryptophan (W), histidine (H), and uracil (U) (SD + L–W–H–U). The selection marker of pEG202 and pJG4-5 vectors is histidine and tryptophan biosynthetic genes, respectively. Also, the reporter plasmid pSH18-34 codes for the uracil biosynthetic gene as a selective marker. Transformants were patched on SD + L–W–H–U. Positive transformants were confirmed by colony PCR with gene-specific primers. To eliminate false positives, EGY48 cells

were transformed with pRFHM1 (an unrelated LexA and *Drosophila* bicoid fusion for DNA binding) as a negative control. Additionally, an empty pEG202 vector was used as a negative control. pSH17-4 (LexA DNA binding and Gal4 activation domain) was transformed into EGY48 cells which served as a positive control.

Leucine prototrophy assay

Leucine prototrophy assay was performed to identify the protein–protein interaction (PPI) via yeast cell growth upon expressing the *LEU2* reporter gene. To evaluate N1a protease–histone and VPg–histone interactions, EGY48 cells transformed with pJGH4–pSH17-4 and pJGH3–pSH17-4 as positive controls, and transformants of pJGH4–pRFHM1, pJGH4–pEG202, pJGH3–pRFHM1, and pJGH3–pEG202 were used as negative controls. Transformants of pEGN1a–pJGH4 and pEGN1a–pJGH3 used for N1a protease–histone interaction evaluation. pEGVPg–pJGH4 and pEGVPg–pJGH3 transformants used to evaluate VPg–histone interaction. To test the activation of the reporter gene (*LEU2*), transformants were grown in SD + L–W–H–U medium up to 0.5 O.D._{600nm} and then subcultured in synthetic dropout medium (SD) with galactose (Gal) and raffinose (Raf) but without leucine (L), tryptophan (W), histidine (H), and uracil (U) (SD + Gal + Raf–L–W–H–U) induction broth with BU salts. The culture was incubated at 30°C for 5 h with shaking at 230 rpm until the cells reached mid-log phase (0.8–1 OD_{600nm}). The cultures were then serially diluted and 10 µl was spotted on the SD + Gal + Raf–L–W–H–U plate. The plates were incubated at 30°C for 2–3 days until colonies appeared.

β -Galactosidase assay

β -Galactosidase plate assay was performed to examine the expression of the *LacZ* reporter gene in pSH18-34 through VPg–H4 and VPg–H3 interactions. Cells were streaked on SD + Gal + Raf + X-gal + L–W–H–U plates. Bait–prey interaction was confirmed by the presence of blue-coloured colonies. Positive and negative controls used as same as leucine prototrophy assay. The β -galactosidase activity was measured as described previously (Lee et al. 2002). The β -galactosidase activity was calculated by the following formula (Miller 1972):

β -galactosidase units

$$= 1000 \times [(OD_{420 \text{ nm}} - 1.75 \times OD_{550 \text{ nm}})] / (t \times V \times OD_{600 \text{ nm}}),$$

where: t = elapsed time (min) of incubation; V = 0.1 ml x concentration factor.

Table 1 List of primers used in this study

Primer name	Sequence
H3Y2H.F	5'CGCCGAATTCATGGCCCGTACGAAGC3'
H3Y2H.R	5'CGCCCTCGAGCTAGGCCCTTTCGCC3'
H4Y2H.F	5'CGCCGAATTCATGTCCGGGCGTGGG3'
H4Y2H.R	5'CGCCCTCGAGCTAGCCGCCGAAGCC3'
NlaY2H.F	5'CCCGAATTCATTGACACAAGGTTGTCCAAC3'
NlaY2H.R	5'GGGCTCGAGTTACTGTATTTTCCTTGGGTCATATCC3'
VPgY2H.F	5'CCCGAATTC AAAGGGAAGCATTGAATAGA3'
VPgY2H.R	5'GGGCTCGAGCTGTATTTTCCTTGGGTCATATCC3'

Results and discussion

ER motif is found in most of the Esa1-related MYST family HAT, which is known to acetylate H3 and H4 histones in the nucleosome (Adachi et al. 2002). Active residues of ER motif are found in Nla proteases of CdMV, Artichoke latent virus (ArLV), and Chinese yam necrotic mosaic virus (CYNMV) (Fig. 1a). The functional role of this putative histone-binding motif of CdMV Nla protease was examined by yeast two hybrid interaction assays. Leucine prototrophy assay helps to identify the bait–prey interaction via yeast cell growth upon expressing the *LEU2* reporter gene (Gol-emis et al. 2001). In this assay, pEGNla protease-pJGH3 and pEGNla protease-pJGH4 harbouring EGY48 yeast cells were not grown on the induction medium of SD + Gal + Raf-L–W–H–U. Positive control pSHL17-4 harbouring cells were grown up to the dilution of 10^{-4} . Cells with negative controls pRFHM1 and empty pEG202 constructs were not grown on the plate (Fig. 1b). It showed that no interaction found between Nla protease with H3 and H4. Observation of putative retinoblastoma related (RBR) protein-binding domain of potyvirus A Nlb did not show any functional activity (Oruetebarria et al. 2002). Similarly, yeast two hybrid interaction assays established that this putative ER motif in CdMV Nla protease does not confer binding to host histones H3 and H4. Moreover, the putative ER motif was conserved only to macluravirus and absent in other genera. Inconsistent distribution of this putative motif in potyvirids is not a functionally valid motif.

Potyvirus Nla protease enters the plant nucleus as VPg–Nla protease amalgamate, and it depends on the nuclear localization signal of VPg (Rajamaki and Valkonen 2009). The interaction assay was performed for CdMV VPg with histones H3 and H4. This interaction was easily detected by the strong growth on stringent selection medium SD + Gal + Raf-L–W–H–U of leucine prototrophy assay. The yeast cells harbouring pEGVPg-pJGH3, and pEGVPg-pJGH4 were grown up to the dilution of 10^{-4} (Fig. 1c). This assay functionality was validated by showing the proper

growth of positive and negative controls. This interaction was further confirmed with *LacZ* reporter activity. Bait–prey interaction was confirmed by the blue colour formation of yeast cells on the selection medium. Expression of reporter protein β -galactosidase was readily detected in VPg–histone (H3 and H4) interactions (Fig. 1d, e). The reporter β -galactosidase activity was quantified by Miller analysis to measure the VPg–histone interaction (Table 2). Quantification of β -galactosidase reported expression showed that VPg–H4 interaction was stronger than VPg–H3.

Conclusion

The present study identified the potential interaction between CdMV VPg protein and cardamom histones H3 and H4. Though CdMV Nla protease possesses putative ER histone-binding motif, no interaction was observed with histones. This study clearly indicates that nuclear entry of VPg protein is not only meant for Nla protease transport, and it can access the host genome through histone interaction. The present study throws light on understanding the mechanism of host genome accessibility of potyviral proteins. This study identified novel interacting host partners of VPg. Further investigations would provide novel insights into the molecular regulations underlying CdMV infection and the intricate role of VPg in evading host defence.

Table 2 Quantitative analysis of CdMV VPg and cardamom histones (H3 and H4) interactions

S. No	DNA-binding constructs	Activation domain constructs	β -galactosidase activity (miller units)
1	pEGVPg	pJGH3	63.9 ± 1.5
2	pEGVPg	pJGH4	64.7 ± 1.9
3	pSH17-4	pJGH3	511.8 ± 12.6
4	pSH17-4	pJGH4	518.2 ± 15.5

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Author contributions SNP and JT designed this study. The experiments were performed by SNP and RS. SNP wrote the initial draft, and RS and JT edited the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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