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Effects of LEF-11 acetylation modification on the regulation of baculovirus infection

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Summary. – Late expression factor 11 (LEF-11) is an essential protein in the regulation of Bombyx mori nucleopolyhedrovirus (BmNPV) DNA replication and late gene expression. Our recent quantitative analysis of protein acetylome revealed for the first time that LEF-11 can be acetylated at one lysine residue (K83) during viral infection, but the underlying mechanism is unclear. The acetylation level for K83 was down-regulated after 36 h post-infection by approximately 30%. To clarify the regulatory function of this modification, overlap PCR was used for site-specific mutagenesis for acetylated (K83Q) or deacetylated (K83R) mimic mutants of LEF-11. The results of viral titration and quantitative polymerase chain reaction showed that after K83 acetylation, budding virion production and the viral genome replication level were significantly upregulated. Meanwhile, the results of yeast two-hybrid (Y2H) system confirmed that K83 deacetylation modification inhibited the interaction between LEF-11 and immediate early gene 1 (IE-1). In conclusion, the acetylation of LEF-11 at K83 might enhance the interaction with IE-1 in the host cell nucleus to promote viral DNA replication, and might be one of the antiviral strategies of the silkworm host. The host inhibits virus proliferation by deacetylating LEF-11.

Keywords: BmNPV; LEF-11; acetylation; virus replication; protein interaction

Introduction

BmNPV belongs to the family *Baculoviridae*, which contains large double-stranded circular DNA (80–180 kbp) and is packaged into baculovirus particles. It mainly infects arthropods and causes diseases, especially of the economically important insect *Bombyx mori* (*Lepidopteran: Bombycidae*). It is reported that the annual loss caused by BmNPV infection accounts for 80% of the loss of biological toxicity (Lei *et al*., 2019). During the process of viral infection, the virus hijacking is an important life process of the host, which affects virus proliferation, such as controlling the host cell cycle process and reshaping the cytoskeleton (Gao *et al*., 2021). The host cells activate cellular processes including DNA damage response, heat shock response, apoptosis and ubiquitin-mediated proteasome degradation to resist viral invasion (Xue *et al*., 2012). Although, the interaction mechanism between the virus and host cells is unclear, it has become a focus of viral disease research.

The late expression factors (LEFs) are a type of early gene expression products that can directly or indirectly regulate the transcription and expression of late and

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Abbreviations: cMNPV = Autographa californica multiple nucleopolyhedrovirus; AD = activation domain; BD = DNA-binding domain; BmNPV = Bombyx mori nucleopolyhedrovirus; IE-1 = immediate early gene 1; KAT = lysine acetyltransferase; LEFs = late expression factors; NEDD4 = neuronal precursor cell-expressed developmentally downregulated 4; PTM = post-translational modification; TBK-1 = TANK-binding kinase 1; Y2H = yeast two-hybrid

very late genes, and so far, a total of 20 LEFs have been identified (Berretta *et al*., 2013; Glocker *et al*., 1993; Hefferon, 2004). *Lef-11* is one of the *lefs*, participating in viral DNA replication and localization in the nucleus. Except for the dipteran Culex nigripalpus nucleopolyhedrovirus (CuniNPV) genome, *lef-11* is present in all baculovirus genomes. *Lef-11* (GenBank: 1403869) of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) was found to be one of the essential genes for viral genome DNA replication and has 97% of amino acid sequence similarity with BmNPV (Lin and Blissard, 2002). The *lef-11* (GenBank: 1488659) gene of BmNPV is 336 bp long and encodes 112 amino acids with a molecular weight of 13.1 kDa, located in the nucleus. Previous studies have reported that the LEF-11 of BmNPV interacts with LEF-3 and forms homo-oligomers with itself. In addition, LEF-11 and IE-1 co-localize in the nucleus. Therefore, LEF-11 is considered to play a key role in the proliferation of BmNPV by regulating viral DNA replication. Our previous proteomic investigation has revealed for the first time that LEF-11 is deacetylated at K83 during BmNPV infection (Hu *et al*., 2018). Although there are several researches on the function of LEF-11, the mechanism of LEF-11 acetylation during viral infection is still unknown.

The acetylation of the ε-amino group of lysine residues in proteins is a widely utilized reversible post-translational modification (PTM) that regulates variety of cellular processes, including chromatin structure, transcriptional regulation, DNA damage repair, cellular metabolism, cytoskeletal dynamics, and apoptosis (Drazic *et al*., 2016; Narita *et al*., 2019). It is controlled by two groups of enzymes: lysine acetyltransferases (KATs) and lysine deacetylases (KDACs) (Gil *et al*., 2017). Proteomic analysis revealed that acetylation occurs in more than 2000 proteins involved in different cellular processes (Choudhary *et al*., 2009). Therefore, lysine acetylation has arisen as an important PTM that competes with phosphorylation and ubiquitination (Choudhary *et al*., 2009; Weinert *et al*., 2011). Since viruses cannot replicate on their own, they have developed unique abilities to alter metabolic and signaling cell pathways, including protein PTMs, to ensure replication of their genomes (Loboda *et al*., 2019). In recent years, more and more studies have revealed that lysine acetylation modification will be exploited for viral replication during infection. Ma *et al*. (2020) have found that influenza virus non-structural protein 1 (NS1) acetylation at K108 has an important role in the replication and virulence. Another study found that Ebola virus (EBOV) ubiquitin ligases NEDD4 is acetylated at a conservative K667 mediated by the acetyltransferase P300, which promotes NEDD4-VP40 interaction and have a physiological effect on EBOV life cycle (Zhang *et al*., 2021). HIV-1 infection augmented the acetylation of microtubules mediated through an interaction with an end-binding protein 1 (EB1) protein and promote the proliferation of HIV-1 (Sabo *et al*., 2013). Interestingly, acetylation modification is a defense mechanism for the host against viruses. TANK-binding kinase 1 (TBK1) is a core kinase of antiviral pathways and activates the production of interferons. It has been reported that TBK1 is deacetylated by histone deacetylase 3 to promote innate antiviral immunity (Tang *et al*., 2021). The acetylation modification of proteins can be regarded as an important means for host defense and virus proliferation. Since no KAT encoding by BmNPV has been found so far, the investigation of the specific function and regulatory mechanism of LEF-11 acetylation modification during the viral infection process will be of great importance, especially, to further understand the interaction mechanism between BmNPV and silkworm host cells.

Materials and Methods

Cells, strains, and plasmids. BmN cells derived from the ovarian tissue of silkworm (*B. mori*) were maintained at 27°C in Sf-900 medium (Thermo Fisher Scientific, USA) with 10% fetal bovine serum (FBS; Corning, USA). *Escherichia coli* strains DH5α, TG1, DH10Bac were stored in our laboratory. The plasmids pFastbac1, pET-28a, pIEx-1, pKD46, pKD3 and BmNPV (Bacmid; Thermo Fisher Scientific, USA) were also maintained in our laboratory. The Yeast two-hybrid plasmids pGADT7 and pGBKT7 were donated by Professor Xijie Guo from Jiangsu University of Science and Technology.

Construction of the lef-11-knockout and repaired Bacmid. To knockout *lef-11* gene, a targeting linear fragment was constructed using PCR utilizing the pKD3 as the template and *lef-11*-ko F and *lef-11*-ko R primers (Table 1). The *lef-11*-ko F and *lef-11*-ko R primers contain a 50 bp homologous sequence of *lef-11* and a 20 bp *cat* homologous sequence. The constructed targeting liner fragment was then transformed into DH10Bac cells that contained the bacmid and were transformed with pKD46. The λ Red recombinase encoded by pKD46 can be induced by *L*-arabinose. The recombinase induces homologous recombination between *lef-11* gene which is located at Bacmid and the targeting linear fragment. The *lef-11*-KO-Bacmid positive clones were screened on LB solid plates containing chloramphenicol, kanamycin and tetracycline. The *lef-11*-KO-Bacmid was confirmed by PCR using *lef-11*-F and *lef-11*-R primers (Fig. 1a). Using wild-type Bacmid (BmBacmid) as template and *lef-11*-F and *lef-11*-R primers, the wild-type *lef-11* gene was cloned by PCR. The *Eco*R I and *Xho* I restriction sites were introduced into the upstream and downstream primers to insert wild-type *lef-11* into pFastBac1. The *lef-11*-R primer also contains a Flag tag (Fig. 1b). The recombinant vectors

Table 1. Primers and sequences used

Primer	Primer sequence (5'→3')
lef-11-ko F	TGCCCCCCAA AAATTGCACG CACTTAGGCG GGTGTAA TTC GGACTGCTTG GTGTAGGCTG GAGCTGCTTC
lef-11-ko R	CTTCTGTATT CATTGTGTGC TTGAGCGTGT TGATGGTT TC CCTGAACAGC ATGGGAATTA GCCATGGTCC
lef-11-F	CCGGAATTCC GTAACAATTG CGAAATAACG CGCAG
lef-11-KQ-R	CTGGCAATTC GTTGGTGATG A
lef-11-KR-R	CTGGCAATTC GCCTGTGATG A
lef-11-KQ-F	TCATCACCAA CGAATTGCCA G
lef-11-KR-F	TCATCACAGG CGAATTGCCA G
lef-11-R *Xho*I	CCGCTCGAGT TACTTGTCAT CGTCGTCTTT GTAGTCCC ATGTTTGATT TTTGTAAAC
M13 F	AGGGTTTTCC CAGTCACG
M13 R	GAGCGGATAA CAATTTCACA C
gp41-F	CGTAGTGGTA GTAATCGCCG C
gp41-R	AGTCGAGTCG CGTCGCTTT
YthS *lef-11 Eco*RI F	CCGGAATTCA TGCCCCCCAA AAATTGC
YthS *lef-11 Bam*HI R	CGCGGATCCT TACCATGTTT GATTTTT
YthS *ie-1 Eco*RI F	CCGGAATTCA TGACGCAAAT TAATTTT
YthS *ie-1 Bam*HI R	CGCGGATCCT TAATTAAATT CAATTTT
YthS *lef-3 Eco*RI F	CCGGAATTCA TGGCGACCAA AAGATTTTTT
YthS *lef-3 Bam*HI R	CGCGGATCCT TACAAAAATG TATAATCATT

Footnotes: Dotted line indicates homologous arms of *lef-11* gene; Wave line indicates enzyme digestion sites; Single line indicates the base introducing the mutation site; Double line indicates Flag tag.

were transformed into DH10Bac competent cells containing *lef-11*-KO-Bacmid that had been prepared before. White colonies grown on LB solid plates containing antibiotics, were selected for PCR detection, and the 3 pairs of primers, M13 F and *lef-11*-R, M13 F and M13 R, M13 R and *lef-11*-F, were used to confirm whether wild-type *lef-11* was repaired. Finally, the repaired Bacmid (*lef-11*-RE-Bacmid) was constructed.

Construction of the lef-11-KQ/R-Bacmid. The site-directed mutations for acetylated (K/Q) or deacetylated (K/R) mimic mutants were generated by gene splicing and overlap extension PCR (Fig. 1c). PCR was performed with wild-type *lef-11* gene as the template and *lef-11*-F and *lef-11*-KQ/R-R, *lef-11*-R and *lef-11*-KQ/R-F primers. Then, PCR products were used as the template, and *lef-11*-F and *lef-11*-R were used as primers to carry out another PCR. PCR products were ligated in to pFastbac1 and transformed into DH10Bac competent cells. Finally, the positive strains on LB solid plates were selected for PCR verification.

Western blot analysis of lef-11 expression. Transfection was performed using FuGENE®6 Transfection reagent (Promega, USA). BmN cells were transfected with Bm-Bacmid, *lef-11*-KO-Bacmid and *lef-11*-RE-Bacmid. After 96 h the cells were harvested and the proteins were extracted using lysis buffer containing protease inhibitor cocktail (Bimake, USA). The prepared samples were then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, USA). Anti-flag polyclonal antibody (Cell Signaling Technology, USA) and goat anti-mouse monoclonal antibody (Biosharp Life Sciences, China) were used for protein detection.

Quantitative PCR analysis of viral DNA replication and titer detection. BmN cells were transfected with 5 kinds of Bacmids (BmBacmid, *lef-11*-KO-Bacmid, *lef-11*-RE-Bacmid, *lef-11*-KQ-Bacmid, *lef-11*-KR-Bacmid) in the logarithmic growth phase. After 72 h, extraction of total DNA by the method of Tris-balanced phenol was performed. In order to remove the exogenous DNA that has been introduced during the transfection, *Dpn* I (Thermo Fisher Scientific, USA) was used to enzymolyze the methylation sites. Finally, the samples were analyzed by qPCR with the progeny viral genomic DNA as the template and *gp41* F and *gp41* R primers. The GoTaq® qPCR Master mix for qPCR analysis was form Promega (USA).

The viral supernatants were diluted from 10^{-1} to 10^{-10}. The BmN cells were seeded into 96-well plates at a density of 1×10^4 cells/well and each serial dilution was inoculated into the wells. Each dilution was repeated 8 times to ensure the accuracy of the data. The viral titer ($TCID_{50}$) was calculated by the Reed and Muench method.

Yeast two-hybrid assay. The *lef-11*, *lef-3* and *ie-1* were cloned (in frame) into plasmids pGADT7 (Gal4 AD) and pGBKT7 (Gal4 BD), and co-transformed into the Y2H reporter strain AH109. Also, pGADT7-T and pGBKT7-53 was used as the positive control and pGADT7-T and pGBKT7-Lam, pGADT7 and pGBKT7, etc. as the negative controls. The plasmid-introduced AH109 were spread on the SD/-Trp/-Leu minus medium and cultured at 28°C. After 72~96 h, white colonies were selected and cultured in SD/-Trp/-Leu/-His/-Ade/X-α-Gal minus medium and cultivated at 28°C for 96~120 h. When, AD-linked proteins and BD-linked proteins interacted, blue colonies were formed on the SD/-Trp/-Leu/-His/-Ade/X- α-Gal minus medium.

Statistical analysis. Each experiment was performed in biological triplicates independently and data were shown as mean ± SD (standard deviation) by student's *t*-test using GraphPad Prism 7.

Results

Construction and identification of lef-11-knockout, repaired and mutant Bacmids

In order to knockout *lef-11*, *cat* was inserted into the *lef-11* gene. The recombinant Bacmid was identified by PCR with *lef-11*-F and *lef-11*-R primers. Nucleic acid electrophoresis results indicated that the length of the amplified product was about 1600 bp, and this was consistent with the theoretical value of 1599 bp (Fig. 2a).

To get the *lef-11*-RE-Bacmid, Bac to Bac system was used. The *lef-11* coding sequence was fused with its own promoter sequence and the corresponding mutants were repaired back to the virus genome. Positive single colonies from the agar plates which have contained gentamicin, kanamycin, tetracycline, IPTG and X-gal were selected. The PCR ampli-

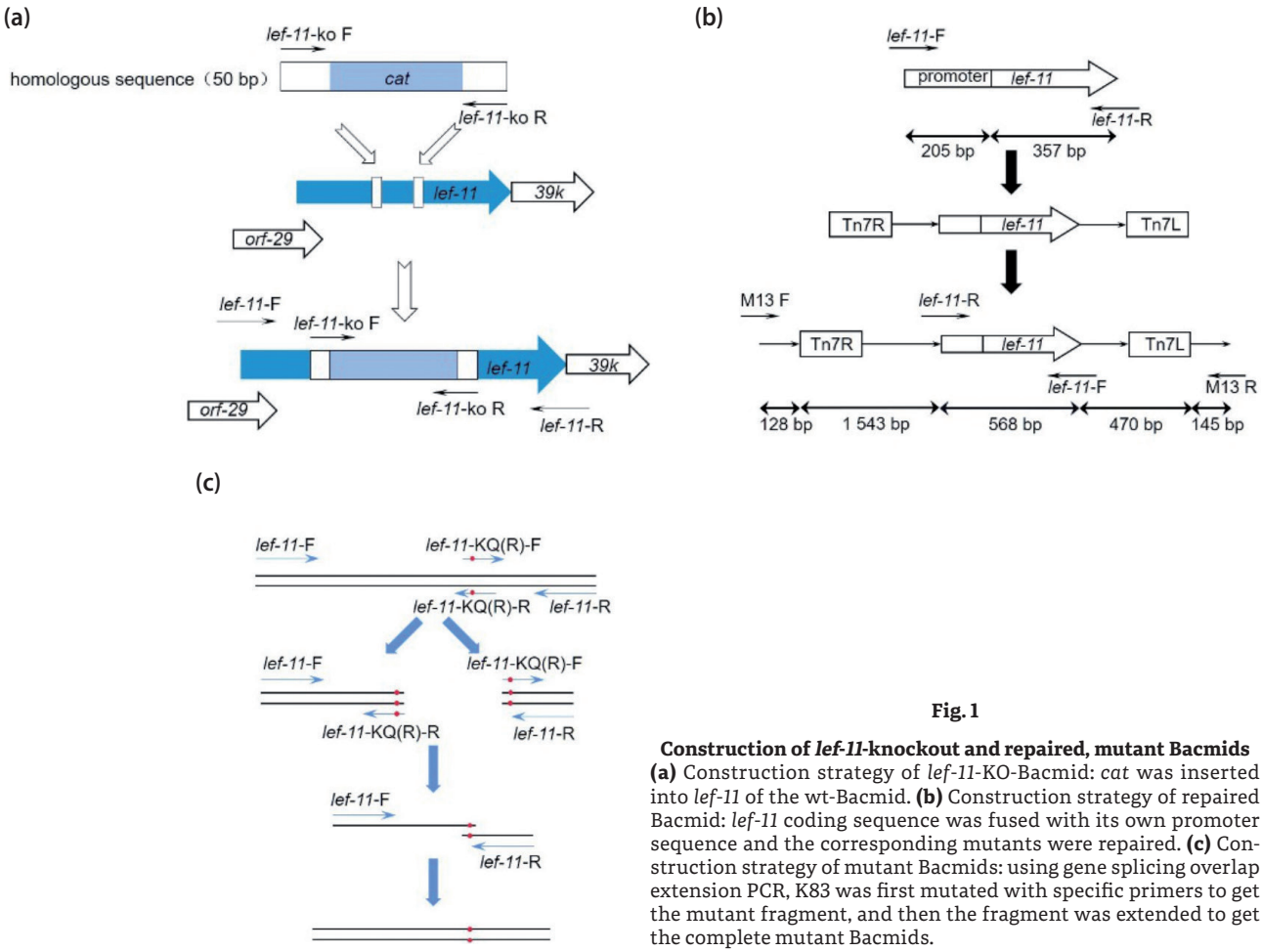


Fig. 1

Construction of *lef-11*-knockout and repaired, mutant Bacmids
(a) Construction strategy of *lef-11*-KO-Bacmid: *cat* was inserted into *lef-11* of the wt-Bacmid. **(b)** Construction strategy of repaired Bacmid: *lef-11* coding sequence was fused with its own promoter sequence and the corresponding mutants were repaired. **(c)** Construction strategy of mutant Bacmids: using gene splicing overlap extension PCR, K83 was first mutated with specific primers to get the mutant fragment, and then the fragment was extended to get the complete mutant Bacmids.

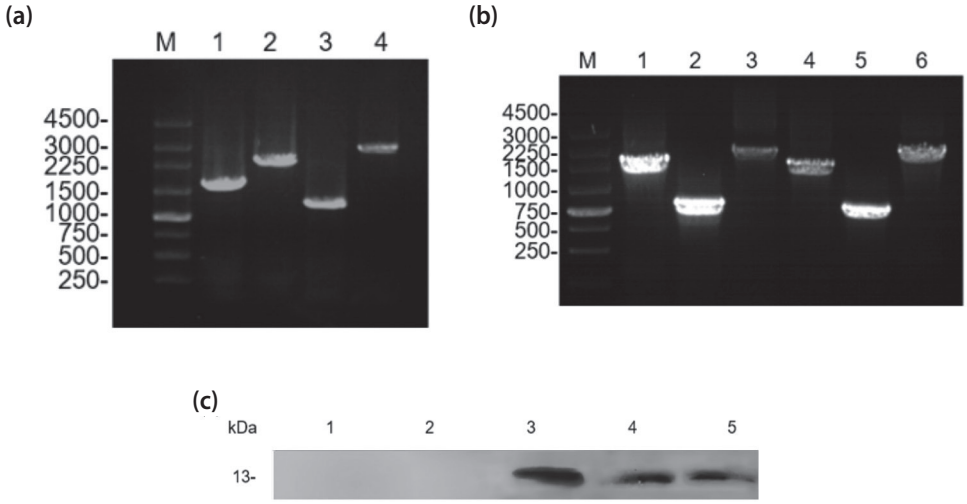


Fig. 2

Identification of *lef-11*-KO and repaired, mutant Bacmids

(a) PCR identification of *lef-11*-KO-Bacmid and *lef-11*-RE-Bacmid. M: molecular weight marker; PCR products of DH10Bac 1: *lef-11*-KO-Bacmid with *lef-11*-F and *lef-11*-R primers; 2: *lef-11*-RE-Bacmid with M13 F and *lef-11*-R primers; 3: *lef-11*-RE-Bacmid with *lef-11*-F and M13 R primers; 4: *lef-11*-RE-Bacmid with M13 F and M13 R primers. **(b)** PCR identification of *lef-11*-KQ-Bacmid and *lef-11*-KR-Bacmid. M: molecular weight marker; PCR products of DH10Bac 1: *lef-11*-KQ-Bacmid with M13 F and *lef-11*-R primers; 2: *lef-11*-KQ-Bacmid with *lef-11*-F and M13 R primers; 3: *lef-11*-KQ-Bacmid with M13 F and M13 R primers; 4: *lef-11*-KR-Bacmid with M13 F and *lef-11*-R *lef-11*-KR-Bacmid with *lef-11*-F and M13 R primers; 6: *lef-11*-KR-Bacmid with M13 F and M13 R primers. **(c)** Polyclonal antibody detection of eukaryotic exogenous LEF-11. Infection with 1: BmBacmid; 2: *lef-11*-KO-Bacmid; 3: *lef-11*-RE-Bacmid; 4: *lef-11*-KQ-Bacmid; 5: *lef-11*-KR-Bacmid.

fied products sizes of about 2250 bp, 1200 bp and 2800 bp were detected, and were consistent with the theoretical sizes of 2239 bp, 1183 bp and 2854 bp, respectively. Also, the mutants were identified by the above PCR method and the nucleic acid electrophoresis results were consistent with the theoretical values (Fig. 2b).

To further confirm that the knocked out, repaired and mutant Bacmids were successfully constructed, western

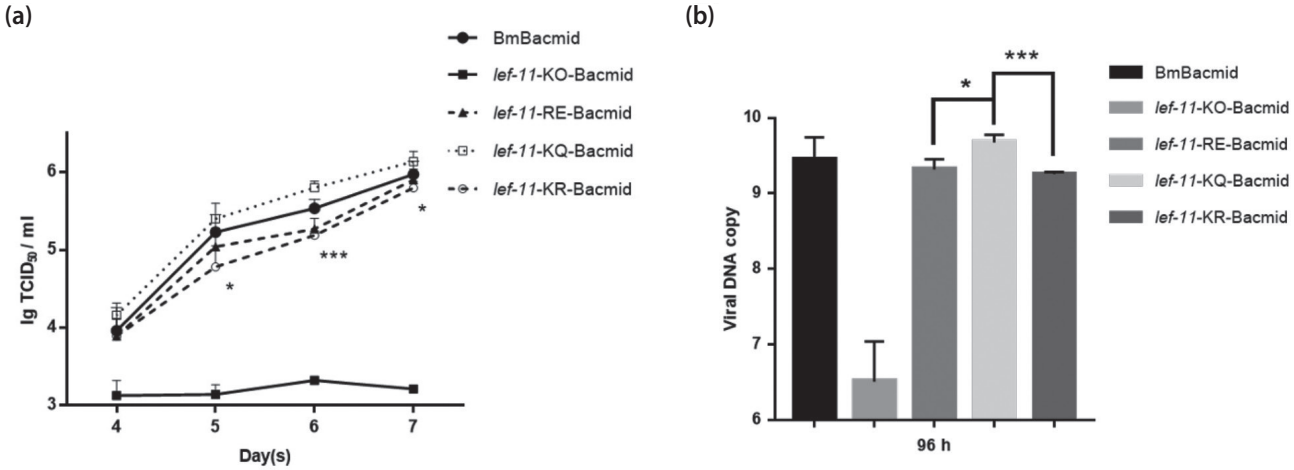


Fig. 3

The effects of acetylated LEF-11 on viral proliferation

(a) BmN cells were transfected with BmBacmid, *lef-11*-KO-Bacmid, *lef-11*-RE-Bacmid, *lef-11*-KQ-Bacmid and *lef-11*-KR-Bacmid. The supernatants were harvested and assessed for virus titers. There was a significant difference between *lef-11*-KQ-Bacmid and *lef-11*-KR-Bacmid ("*" $p < 0.05$, "***" $p < 0.001$). **(b)** BmN cells in the logarithmic growth phase were transfected with 5 kinds of Bacmids (BmBacmid, *lef-11*-KO-Bacmid, *lef-11*-RE-Bacmid, *lef-11*-KQ-Bacmid, *lef-11*-KR-Bacmid), and the total DNA was extracted after 96 h and analyzed for *gp41* by RT-qPCR for viral genome copy number.

blotting was used to identify the expression of *lef-11*. For the BmN cell infection, BmBacmid was used as control. The *lef-11*-KO-Bacmid, *lef-11*-RE-Bacmid, *lef-11*-KQ-Bacmid and *lef-11*-KR-Bacmid were transfected into the cells. As expected, the results showed that the expression of LEF-11 was successful in *lef-11*-RE-Bacmid, *lef-11*-KQ-Bacmid and *lef-11*-KR-Bacmid groups. Since BmBacmid group has no Flag tag and *lef-11*-KO-Bacmid group lacks *lef-11*, there were no bands visible (Fig. 2c).

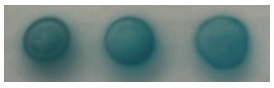
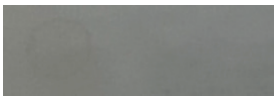
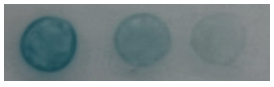
LEF-11 acetylation promotes viral production

To identify the function of LEF-11 acetylation during the process of BmNPV infection, the BmN cells were transfected with BmBacmid, *lef-11*-KO-Bacmid, *lef-11*-RE-Bacmid, *lef-11*-KQ-Bacmid, *lef-11*-KR-Bacmid and analyzed at 96 h post-transfection. The results of quantitative PCR analysis showed that the *lef-11* is essential for viral DNA replication, otherwise they would be inhibited after knockout *lef-11* (Fig. 3b). Meanwhile, the acetylation-mimicking K83 site (Group *lef-11*-KQ-Bacmid) showed significant effect on the viral DNA replication level when compared with others ($p < 0.05$), suggesting that LEF-11 acetylation could promote viral DNA replication. The results of titer detection also showed that acetylation of LEF-11 could increase the ability of BmNPV to generate the budding virions (Fig. 3a).

Deacetylation affects the interaction of LEF-11 with IE-1

It has been reported that acetylation could affect the interaction of proteins. To further explore the protein interaction mechanism of LEF-11 acetylation during the process of BmNPV infection, Y2H system was used to verify

Table 2. Analysis of the interactions among LEF-11, IE-1 and LEF-3

pGADT7	pGBKT7	SD/-Trp/-Leu	SD/-Trp/-Leu/-His/-Ade/X-α-Gal			Interaction	
			1:10	1:100	1:1000		
T	53					yes	positive
T	Lam					no	negative
-	-					no	negative
ie-1	-					no	negative
ie-1	*lef-11*-RE					yes	
ie-1	*lef-11*-KQ					yes	
ie-1	*lef-11*-KR					no	test
lef-11-RE	*lef-11*-RE					no	
lef-11-RE	*lef-3*					no	

Footnotes: The samples were selected by SD/-Trp/-Leu minus medium, and then verified by SD/-Trp/-Leu/His/-Ade/X-α-Gal minus medium. All samples were cultured at 28°C for 72 h.

the interaction of LEF-11, LEF-3, IE-1 and LEF-11 itself. The results showed that only LEF-11 could interact with IE-1. Therefore, we focused on the interaction between LEF-11 and IE-1 when LEF-11 K83 was modified by acetylation or deacetylation (K362Q/R). The development of blue colonies indicated the interaction between K83Q and IE-1, while no interaction relationship was shown between K83R and IE-1, indicating that the K83 deacetylation of LEF-11 might inhibit the interaction with IE-1 (Table 2).

Discussion

Silkworm and baculovirus comprise a well-established model of insect-virus interactions. The interaction mechanism between them has always been a popular topic in the field of virology. However, there are few reports about the post-transcriptional protein acetylation modification involved in the regulation of baculovirus invasion. *Lef-11* is a late expression factor of baculovirus, participating in viral DNA replication and activation of late/very late genes (Chen *et al*., 2017). Our previous acetylome analysis showed that the level of acetylation at LEF-11 K83 was significantly down-regulated post-infection, but the underlying mechanism remains unknow.

In this study, the functional role of LEF-11 acetylation was gained by mutation of lysine residues to glutamine or arginine to mimic acetylation or deacetylation, respectively (Choudhary *et al*., 2009). In theory, conversions to glutamine should induce a different phenotype than arginine substitution, based on the hypothesis that acetylation neutralizes the positive charge of lysins and thus affects various aspects of protein function. However, due to the missense mutation (K to Q/R), the effect of the mutation on the function and structure of the protein cannot be ruled out. Such a phenomenon has also appeared in other acetylation studies and it is the limiting condition for the current studies of protein acetylation (Zhang *et al*., 1998; Giese *et al*., 2017). In our study, there was no significant effect between the mutation of deacetylation (K83R) and control group (Bm) on viral DNA replication or viral proliferation, suggesting that the mutation did not have a significant effect on the viral infection. The K83Q mutation had more positive effects on DNA replication and viral proliferation than control group. Therefore, we speculate that acetylation of LEF-11 at K83 might promote viral proliferation.

As an essential gene for viral DNA replication, knockout of *lef-11* will result in the stagnation of viral proliferation (Lin *et al*., 2002; Li *et al*., 2019). LEF-11 can self-interact to form homo-oligomers (dimers/tetramers), which is one of the necessary conditions for its function (Dong *et al*., 2015). It is common in baculoviruses that the proteins function in homo-oligomers. Activated form of homo-oligomers is a DNA replication-specific expression factor (Morr *et al*., 1995; Downie *et al*., 2013). It has been reported that homo-oligomers are necessary for viral DNA replication during AcMNPV proliferation, including *lef-1*, *lef-2*, *ie-1*, *lef-5*, *dbp*, *ha44* and *cg30* (Hefferon, 2003; Mikhailov *et al*., 2008; Mikhailov *et al*., 2006; Kingsley *et al*., 1999; Kremer *et al*., 1998; Carstens *et al*., 2002; Evans *et al*., 1997). Chen *et al*. (2017) found that LEF-11 of BmNPV interacts with IE-1 and LEF-3 during the infection cycle. We used Y2H system to seek the effect of LEF-11 acetylation on their interactions. The results suggested that K83 deacetylation (K83R) inhibited the interaction of LEF-11 and IE-1. Interestingly, the Y2H results also showed there was no interaction between LEF-11 and LEF-3, or LEF-11 itself. We speculated that their interactions may be too weak to be detected by the Y2H system. For the Y2H assay, the yeast strain used in this study was AH109. This strain carries deletions of the *gal4* and *gal80* genes to avoid interference by endogenous GAL4 and GAL80 proteins during the assay. The nutritional reporter genes ADE2 (coding phosphor-ribosylaminoimidazole carboxylase, an enzyme important for the synthesis of adenine) and HIS3 (encoding imidazoleglycerol-phosphate dehydratase, a key enzyme for histidine biosynthesis) are present in the AH109 strain and allow control of the stringency of selection and reduce the incidence of false positivity.

IE-1 is one of the two trans-regulatory factors encoded by the virus and plays an important role in viral replication, which accelerates the expression of early and late virus genes. Also, IE-1 promotes viral DNA replication by interaction with enhancer sequences (Kawasaki *et al*., 2004; Choi and Guarino, 1995). LEF-3 is located in the nucleus, as a DNA single-strand-binding protein (SSBP), it participates in the regulation of viral DNA replication and late gene transcription. It can also interact with other viral proteins, such as viral helicase P143 and alkaline nuclease (AN), affecting their functions and nuclear localization (Au *et al*., 2009; Mikhailov *et al*., 2003; Kool *et al*., 1994). Therefore, we speculated that the acetylation of LEF-11 K83 is beneficial for LEF-11, IE-1 and LEF-3 complex. IE-1 localizes in the nucleus and participates in the activation of LEF-11 and LEF-3. After the LEF-11 is synthesized in the cytoplasm, it can form dimers/tetramers in the cytoplasm and then bind directly to the host importin α-3 protein. Meanwhile, LEF-11 and LEF-3 form a complex that enters the nucleus and binds to IE-1 (Chen *et al*., 2017). The DNA replication and proliferation of BmNPV were up-regulated. In BmNPV infected BmN cells, the host cells down-regulated the level of acetylation at LEF-11 K83 to inhibit the interaction with IE-1. The complex is one of the conditions under which they function. The viruses do not encode enzymes for dea-

cetylation, so this is most likely a defense mechanism of host cells against viral infection.

There are many reports about acetylation affecting protein interactions and promoting viral proliferation. Romeo *et al.* (2015) found that the P30(II) protein of human T-cell leukemia retrovirus type 1 (HTLV-1) interacts with the oncoprotein c-MYC and enhances c-MYC-dependent transcription and carcinogenesis, and acetylation of c-MYC is a prerequisite for the synergistic effect of P30 (II)/c-MYC compound. Huo *et al.* (2011) found that human immunodeficiency virus 1 (HIV-1) trans-activator protein Tat acetylated at K28 can activate Tat activity and enhance its ability to stabilize microtubules, thereby enhancing the reverse transcription activity of HIV-1 and promoting viral proliferation. Our results showed that LEF-11 acetylation was beneficial for viral proliferation and deacetylation was not conducive to the interaction with IE-1. In the process of BmNPV infection, it first hijacks a host-encoded acetylase to acetylate LEF-11, forming a complex with IE-1 and LEF-3 (Lei *et al.*, 2019; Dong *et al.*, 2015). Then the viral DNA replication starts after forming a replication complex. The host cell responds rapidly, including the production of deacetylating enzymes to deacetylate viral proteins (Lei *et al.*, 2019; Gao *et al.*, 2021). Deacetylation of LEF-11 results in dissociation of the complex. Viral DNA replication and viral proliferation are inhibited. But the results of qPCR and viral titers showed that deacetylated LEF-11 did not inhibit viral proliferation significantly, suggesting that there may be some more complex mechanisms to counteract the adverse effects of LEF-11 deacetylation. However, this hypothesis needs further verification in the future.

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