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Acetylation of late expression factor 4 is crucial for the transcription and proliferation of *Bombyx mori* nucleopolyhedrovirus

Jonas Ngowo[1,2], Xu Gao[1,2], Yajie Zhu[1,2], Fuxiang Mao[1,2], Jihai Lei[1,2], Enoch Obeng[1,2], Wei Yu[1,2*]

[1]College of Life Sciences & Medicine, Zhejiang Sci-Tech University, Zhejiang Province, Hangzhou 310018, P. R. China;
[2]Zhejiang Provincial Key Laboratory of Silkworm Bioreactor and Biomedicine, Zhejiang Province, Hangzhou 310018, P. R. China

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Summary. – Late expression factor 4 (LEF4), RNA polymerase subunit of *Bombyx mori* nucleopolyhedrovirus (BmNPV), plays an enzymatic role to enhance the capping of pre-mRNA of late and very late genes. Lysine acetylation is a post-translational modification process having many important functions associated with the regulation of a gene expression. Our previous study on lysine acetylome in BmNPV infected BmN cells showed that LEF 4 was acetylated at lysine 76 (K76). However, it is still unclear whether the modification of K76 residue contributes to the modulation of viral gene transcription. To elucidate the role played by acetylation or deacetylation of LEF4 K76 in the transcription of viral genes, we constructed acetylation mimicking and deacetylation mimicking mutant virus, K76Q and K76R, respectively. We then transfected BmN cells with these mutants and analyzed the level of pre-mRNA at different times. The K76R showed a significant decrease in the mRNA transcription level of *vp39* and *p10* genes at 48 and 72 h post-transfection, while K76Q did not show any significant changes compared with *lef*4-Wt. We further detected the virus titer of *lef*4-Wt, K76Q & K76R, and it was found that K76R impaired the virus infectivity ability at 72 and 96 h, while K76Q did not affect the virus infectivity. Moreover, the yeast two hybrid technique (Y2H) showed that both mutants (K76Q & K76R) affected the association of LEF 4 with the P47 protein. Taken together, these results indicated that acetylation modification of K76 is important for the proper transcription of late and very late genes, and the effectiveness of viral infection.

Keywords: BmNPV *lef*4 gene; lysine acetylation; late genes transcription; BmNPV *p47* gene; infectivity

Introduction

Baculoviruses are double-stranded DNA viruses with a genome size between 80 to 180 kb. Among these viruses is the *Bombyx mori* nucleopolyhedrovirus (BmNPV), which is known to be one of the pathogens that pose an infection in the silkworm. The BmNPV happens to be the cause of about 20% decrease in sericulture production (Jiang *et al.*, 2013; Zhou *et al.*, 2016). However, the interaction mechanism between the BmNPV and its host (silkworm) remains unclear, and this has been one of the research hotspots of insect virologists in the past years. Moreover, baculovirus has become a useful model for studies and production of recombinant proteins in different cell lines in recent years (Lackner *et al.*, 2008). Therefore, it has multiple values to study the interaction mechanism between virus and insect cells.

The baculovirus life cycle is divided into three different phases: early, late, and very late. In the early phase, most of the genes responsible for viral DNA replication are transcribed, while a number of structural genes and late expression factors (LEFs) are transcribed during the late and very late phases. Unlike other DNA viruses, baculoviruses use the host RNA polymerase II and viral

*Corresponding author. E-mail: mikkyu@163.com; phone: +86-571-86843338.

Abbreviations: AcMNPV = *Autographa californica* multicapsid nucleopolyhedrovirus; BmNPV = *Bombyx mori* nucleopolyhedrovirus; DDO = double drop out; hpt = hours post transfection; LEF = late expression factor; QDO/X = quadruple drop out; Y2H = yeast two hybrid technique

RNA polymerase II to transcribe their early genes, the late and very late genes, respectively (Guarino and Jin, 1998). The baculovirus RNA polymerase II is reported to be the simplest DNA-directed RNA polymerase due to the fact that it is composed of four subunits: LEF4, LEF8, LEF9 and P47 (Glocker *et al.*, 1993).

LEF4, one of the baculoviral RNA polymerase II subunits, is reported to be responsible for mRNA capping mechanism. LEF4 has guanyl transferase activity, which enhances the catalysis of Guanyl Triphosphate (GTP) to form diphosphate (GDP), hence, terminating the 5' mRNA during capping reaction (Guarino and Jin, 1998), and subsequently the hydrolysis of γ-adenosine triphosphate (ATP) to adenosine diphosphate (ADP) at the 5' mRNA end (Gross and Shuman, 1998). The studies indicate that the major role of the LEF4 is the formation of m7G cap at the 5'ends of mRNA of viral late genes, thus preventing transcription products from exonuclease degradation, enhancing the translation process and facilitating the export of mRNA to the cytoplasm for viral protein synthesis (Rohrmann, 2019). Also, LEF4 is required to promote the expression of the late and very late genes, and therefore, the deletion of the *lef4* gene will result in decreased virus progeny production (Knebel-mo *et al.*, 2006).

Posttranslational modification (PTM) is a mechanism of covalent modification of proteins, resulting in change of the properties of a particular protein either through proteolytic cleavage, addition or modification of amino acid residue (Khoury *et al.*, 2011). PTM include acetylation, phosphorylation, glycosylation, methylation, proteolytic cleavage, GPI anchor palmitoylation, ubiquitination, and sumoylation (Biosyst *et al.*, 2012). Recently, acetylation has emerged as a vital PTM that plays important role in the regulation of gene expression (Berro *et al.*, 2006), where a change in lysine conformation results in the removal of unfavorable charges on the amino acid interface and the hydrophobic side chain that retain the intrinsic hydrogen bonding capacity, thereby increasing the DNA binding activity, which leads to the promotion of transcriptional mechanism (D'Orso and Frankel, 2009).

Based on our previous results (Hu *et al.*, 2015), lysine acetylation site (Kac) was identified in BmNPV LEF4 at K76, suggesting that acetylation may have many important roles upon the viral infection. Several studies have reported the impact of acetylation modification on different cellular processes such as gene regulation, DNA replication, and virus infectivity (Berro *et al.*, 2006; Kuczynska *et al.*, 2016; Ma *et al.*, 2020). In this current study, the possible function of the LEF4 K76 acetylation was explored during BmNPV infection. This work will enhance our understanding of the mechanisms of molecular interactions between BmNPV and silkworm hosts.

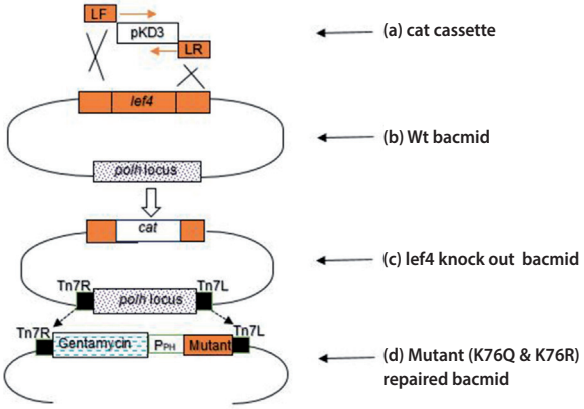


Fig. 1

Knockout and repair strategy of *lef4* gene and mutant (K76Q & K76R) bacmid

(a) *cat* cassette: pKD3 amplified by L-F and L-R primers, which are flanked by pkD3 internally and externally, respectively. **(b)** *lef4*-Wt bacmid; the bacmid containing *lef4* gene in its location. **(c)** *lef4*-Ko; *lef4* gene replaced with cat cassette. **(d)** Mutant (K76Q & K76R) repaired bacmid; Transposition between Tn7 element of recombinant *lef4*-pFastBac and att Tn7 site on knock out bacmid.

Materials and Methods

Cell line, viruses, and culture techniques. BmN cells derived from the ovaries of silkworms were collected and maintained in our laboratory. Cells were cultured at 27°C in SF900 medium (Therma Fisher Scientific, USA) supplemented with 10% fetal bovine serum (Gibco Company, USA). To construct *lef4*-deficient bacmid, λ recombinase technique was employed with two primers L-F and L-R, which were internally overlapped with chloramphenicol cassette, pKD3 gene and externally flanked by *lef4* gene (Table1). Primers were designed and the product amplified using pKD3 as a plasmid template to produce a linear fragment containing gene cassette (*cat*) (Fig. 1a). The competent DH10Bac *Escherichia coli cells* containing pKD46 induced by 2 M *L*-Arabinose were transformed with the linear fragment (*cat*-cassette), incubated at 37°C in a shaker at 220 rpm for 1.5 h. The inoculum was then spread on LB solid medium containing 50 μg/ml chloramphenicol, 50 μg/ml kanamycin, and 50 μg/ml tetracycline. After 72 h, clones were selected from the plate and re-cultured. In the construction of the mutants, a three PCR reaction system was employed. The 1st and 2nd fragments of both mutants were formed in the first and second PCR reaction system respectively, using primers: *lef4*-F & 76R-R (1st fragment) and *lef4*-R & 76R-F (2nd fragment) for the positive mutant, *lef4*-F & 76Q-R (1st fragment) and *lef4*-R & 76Q-F (2nd fragment) for the negative mutant. Both fragments of either mutant were then fused in a third PCR reactions system using *lef*-4-Wt primers (*lef4*-F & *lef4*-R), to obtain the positive (K76Q) and negative (K76R) mutant, respectively. To generate *lef4* Repaired (*lef4*-Re) bacmid, the *lef4* recombinant pFastBac (*lef4*-pFastbac) plasmid

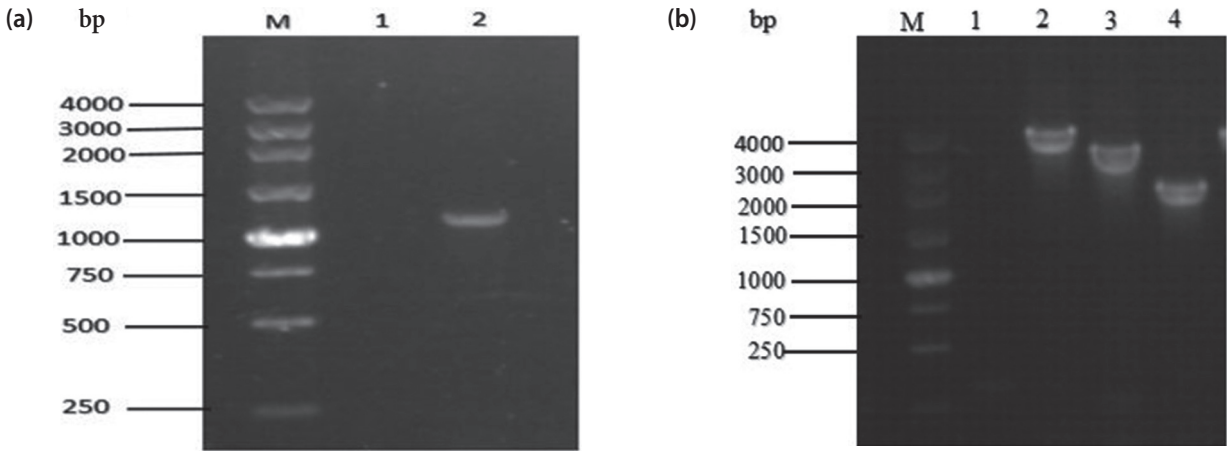


Fig. 2

The PCR products for confirmation of *lef4* knockout, repaired and mutant (K76Q & K76R) bacmid
(a) Lane1: the *lef4*-Wt (*lef4*-F & *lef4*-R) primers were used for amplification; Lane2: the cat primers (L-F & L-R) that are flanked by pkD3 internally and externally, respectively, were used for PCR confirmation. **(b)** Lane1: Blank control (water); Lane 2: the confirmation of repaired bacmid using M13-F & M13-R primers; Lane3: M13-F & LEF4-R primers; Lane4: M13-R & LEF4-F primers.

was constructed through the insertion of the *lef4* gene into the plasmid downstream of the polyhedron promoter. Bac-to-Bac Baculovirus system was then used to transform recombinant *lef4*-pFastbac into DH10bac *E. coli* containing linear fragment (*lef4* deficient) and plated on solid LB medium containing Tetracycline, Kanamycin, gentamycin, IPTG, and 5-Bromo-4-chloro-3-indole-B-d-galactopyranoside (x-gal) (Fig. 1b,c,d). K76Q and K76R were also constructed using the aforementioned Bac-to-Bac system. Finally, the *lef4* (F and R) primers together with M13 primers were used for PCR confirmation of *lef4*-Re (Fig. 2b).

Virus titer detection. BmN cells were transfected with *lef4* Wildtype (*lef4*-Wt), *lef4*-Knockout (*lef4*-Ko), K76Q and K76R bacmids and incubated at 27°C for 72 h to generate the first viral generation, i.e.: *lef4*-Wt, *lef4*-Ko, K76Q, and K76R virus, respectively. Supernatants were collected and stored at 4 °C. Cells were seeded in a 96-well plate at a concentration $1x10^4$ cells/well, incubated at 27°C overnight to allow the cells to adhere to the plate and form a monolayer. A serial dilution of the c ollected supernatant was prepared from 10^{-1} to 10^{-9}, followed by inoculation of 10 µl of each dilution into 10 wells and incubated at 27°C for 5 days. After the 5th day, the number of infected wells for each serial dilution was recorded and used to calculate viral titers ($TCID_{50}$) using Reed-Muench method (Reed and Muench, 1938). The experiment was repeated at different time points 18, 24, 48, 72 and 96 h.

qPCR assay of viral gene transcription level in different phases of infection. The BmN cells were transfected with 1 µg of *lef4*-Wt, K76Q and K76R bacmids. At 24, 48 and 72 h, the cells were harvested and their total RNA was extracted using Trizol reagent. DNase I was used to eliminate any traces of genomic DNA residues. Afterward, the synthesis of cDNA was done by the aid of reverse transcriptase and oligo dT. Designed specific primers of the *lef3, vp39*, and *p10* genes, as shown in Table 1,

Table 1. List of primer sequences

Primer name	Primer sequence
lef4-F	5'-CCGGAATTCTTTTTGACATTTTAAAACG-3'
lef4-R	5'-CCCAAGCTTTTAGTGGTGATGGTGATGA TGATTTGGCACGATTC-3'
76R-F	5'-AAACACAAAAGATTTGTTTATTGG-3'
76R-R	5'-CCAATAAACAAATCTTTTGTGTTT-3'
76Q-F	5'-AAACACAAACAATTTGTTTATTGG-3'
76Q-R	5'-CCAATAAACAAATTGTTTGTGTTT-3'
L-F	5'-CACCATTCGTGCCGCTGACCACGTTG ATTGACAAGATGGCCCCTCG AAAAGTG TAGGCTGGAGCTGCTTC-3'
L-R	5'-TTTTACGGACGCGTTGTCGAACGCGTCGTCT CCGTACACTATTT TTTGTTATGGGAATTAGCCA TGGTCC-3'
M13-F	5'-CCCAGTCACGACGTTGTAAAACG-3'
M13-R	5'-AGCGGATAACAATTTCACACAGG-3'
lef3-F	5'-TCGGATGACCGTTCTACCTCTT-3'
lef3-R	5'-CTTCCAGCAGCATTGAGATTTG-3'
vp39-F	5'-AGACACCACAAACCCGAACAC-3'
vp39-R	5'-TTGATCGCCAACACCACCT-3'
p10-F	5'-TTTAGACGCCATTGCGGAAAC-3'
p10-R	5'-CGATTCTTCCAGCCCGTTT-3'
β-*Actin*-F	5'-GCGCGGCTACTCGTTCACTACC-3'
β-*Actin*-R	5'-TGCCGCAAGCTTCCATACCC-3'

All site directed mutageneses were verified by PCR, Double Digestion and DNA Sequencing (Sangon sequencing Company, Shanghai). The underlined label represents the base pairs for the site directed mutation and the underlined label represents the upstream and downstream arms for the knocked-out site of the gene.

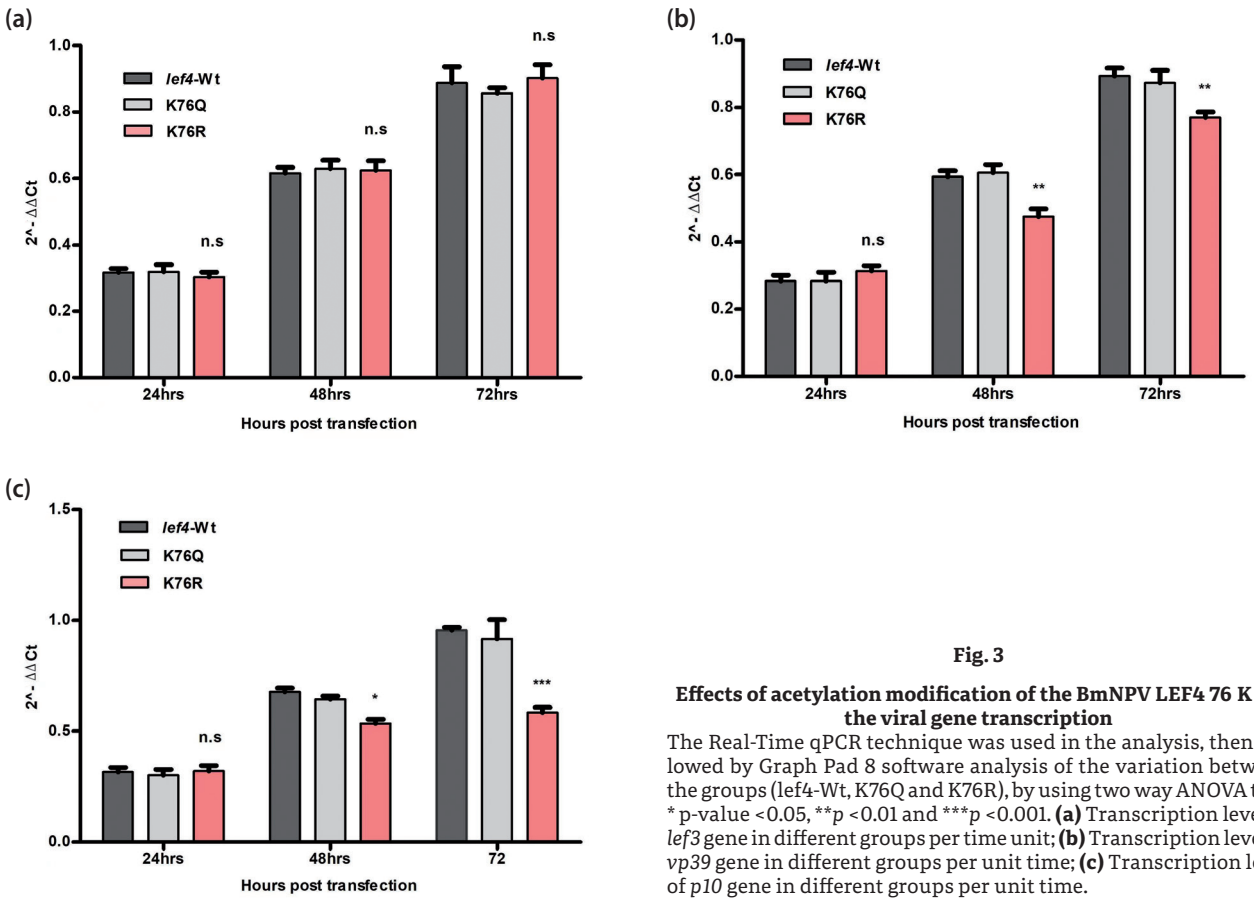


Fig. 3

Effects of acetylation modification of the BmNPV LEF4 76 K on the viral gene transcription

The Real-Time qPCR technique was used in the analysis, then followed by Graph Pad 8 software analysis of the variation between the groups (lef4-Wt, K76Q and K76R), by using two way ANOVA test, * p-value <0.05, **p <0.01 and ***p <0.001. **(a)** Transcription level of *lef3* gene in different groups per time unit; **(b)** Transcription level of *vp39* gene in different groups per unit time; **(c)** Transcription level of *p10* gene in different groups per unit time.

were used to amplify synthesized cDNA in different samples (*lef4*-Wt, K76Q, and K76R). Maxima SYBR Green qPCR Master Mix (2×) containing SYBR Green I dye was added to enable DNA detection and analysis. β-actin was used as an endogenous control gene to normalize the target gene expression level. Finally, the sample mixtures were analyzed by Applied Bio systems 7500 Real-Time PCR System and using 7500 software v2.3 and relative gene expression using delta delta Ct method (2–ΔΔCt).

Yeast two-hybrid technique (Y2H). The BmNPV *lef4*-Wt and the mutants, K76Q, K76R, were all constructed into pGBKT7 DNA-BD vector, while BmNPV *p47* was constructed into pGADT7 AD vector. Next, the *lef4*-Wt and the two mutants were independently co-transformed with *p47* using AH109 yeast competent cells (Weidi Biotechnology, Shanghai China) and plated on different plates that lacked both leucine and tryptophan, which is known as double drop out (DDO) medium. Concurrently, AH109 yeast cells were also co-transformed with the recombinant vectors, pGBKT7-*p53* & pGADT7-*T* and pGBKT7-*Lam* & pGADT7- *T,* to serve as a positive and negative control, respectively. Afterward, all the plates were incubated at 30 °C for 48 h. Colonies were selected and re-cultured on the quadruple drop out (QDO/X/A) plate, which lacked leucine, tryptophan, histidine, and adenine but contained chromogenic substrate (X-α-gal). The QDO plate was then incubated at 30°C for 72 h and data were collected.

Data analysis. Data were analyzed using Graph Pad Prism version 8 (Graph Pad software). Each experiment was performed in triplicate independently. A two-way ANOVA statistical test was used to determine the comparison between groups, and the level of significance is indicated as significant when p <0.05 and very significant (p <0.01).

Results

Confirmation of lef4 knockout, repaired and mutant bacmid

The cells were transformed with *lef4* Knockout (*lef4*-Ko), *lef4*-Re, K76Q and K76R bacmids and then plated on a solid medium containing chloramphenicol, tetracycline, and kanamycin antibiotics. Following the overnight culturing of cells, clones were randomly picked from the plate, and re-cultured. The *lef4*-Ko, *lef4*-Wt and cat primers

(L-F & L-R) primers were used for PCR amplification with *lef4*-Ko as a template. The theoretical PCR product size of the *lef4*-Wt and cat primers were 2732 bp and 1132 bp, respectively. The size of the bands obtained was similar to the expected band size. This result indicated that *lef4* gene was successfully knocked out (Fig. 2a). To confirm whether the repairing of the *lef4* gene was successful, the M13 primers together with the wild type primers (*lef4*-F & *lef4*-R) were used for PCR analysis, *lef4*-Re, K76Q and K76R were used as DNA templates. Using (M13-R & M13-F), (M13-F & *lef4*-F) and (M13-R & *lef4*-F) primers in a PCR reaction resulted in PCR products with sizes of about 3900 bp, 3000 bp and 2400 bp, confirming the *lef4*-Re (Fig. 2b). These results demonstrated that the *lef4* gene was knocked out and repaired successfully.

Effects of acetylation modification of the BmNPV LEF4 K76 on the transcription of viral genes

To elucidate the effect of acetylated LEF4 on the transcription of early genes, we measured the mRNA level of *lef3* in the cells transfected with *lef4*-Wt, K76Q and K76R bacmids. The results showed no difference in *lef3* mRNA level in cells transfected with *lef4*-Wt, K76Q or K76R (Fig. 3a). However, the cells transfected with *lef4*-Ko were unable to produce infectious virions. These results indicated that the acetylation or deacetylation of K76 in the LEF4 had no effect on the transcription of early genes. To study the impact of lysine acetylation of LEF4 on the late and very late genes expression, we determined the level of transcripts of *vp39* and *p10* genes in the BmN cells transfected with *lef4*-Wt, K76Q, and K76R bacmids. Cells transfected with *lef4*-Wt and K76Q bacmids did not show a significant difference in the expression level of both *vp39* and *p10*, which are the late and very late genes, respectively; these results indicated that the change of lysine to glutamine does not attenuate transcription of late gene, instead, it reveals the constant mimicking of the *lef4*-Wt. On the other hand, the K76R showed a substantial decrease of *vp39* and *p10* levels at 48 and 72 hours post transfection (hpt) compared to *lef4*-Wt and K76Q (Fig. 3b,c). This suggested that the replacement of lysine amino acid residue at K76 with arginine (R) residue disrupts the late gene transcriptional process. Therefore, these results indicated that the acetylated LEF4 K76 is important for the expression of viral late and very late genes.

Acetylation of LEF4 moderates the reproduction of BmNPV

To determine the effects of acetylated LEF4 K76 on the virus infectivity, the cells were infected with generated viruses of *lef4*-Wt, *lef4*-Ko, K76Q, and K76R and the virus titer was analyzed. Following the infection of the BmN cells, *lef4*-ko virus did not cause any infection. These results strongly suggest that the *lef4* gene is very crucial for virus viability. Some difference was observed between *lef4*-Wt and K76Q virus. Conversely, K76R significantly affected the virus infection ability at 24, 72, and 96 hours post infection, compared with the other two viruses (*lef4*-Wt & K76Q). Consistent with transcriptional levels results, these results strongly suggested that acetylation of K76 is required for the normal reproduction function of the virus (Fig. 4).

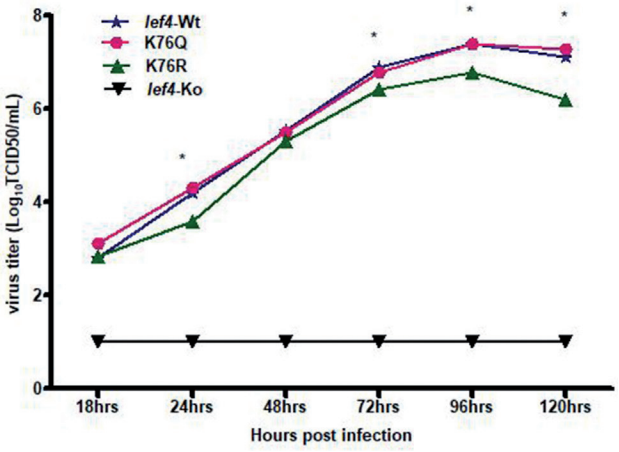
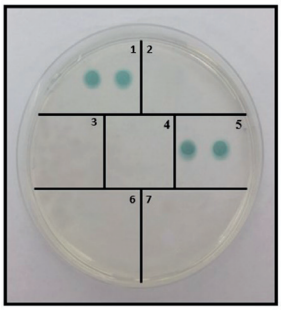


Fig. 4

Acetylation of LEF4 moderates the normal function of the virus
The BmN cells were infected with *lef4*-Wt, *lef4*- Ko, K76Q and K76R generated viruses, and at different time points virus titers were analyzed

The effects of lysine acetylation of BmNPV-LEF4 K76 on its association with BmNPV-P47

The interaction of AcMNPV-LEF4 with AcMNPV-P47 was initially described based on co-immunoprecipitation assay (Co-IP), reporting the association between these two proteins (Crouch, *et al.*, 2007). Therefore, we decided to probe the effects of BmNPV-LEF4 modification on its interaction with the partner BmNPV-P47, through the Yeast 2 Hybrid (Y2H) technique. BmNPV *lef4*-Wt, K76Q and K76R were constructed into pGBKT7 plasmid, and BmNPV-p47 into pGADT7 plasmid. These plasmids were then co-transformed into yeast cells, cells were plated onto DDO plates and further re-cultured on QDO plates. The presence of blue colonies indicated that there was an interaction between LEF4 and P47. Intriguingly, neither K76Q nor K76R mutant showed blue colonies, which suggested that there was no interaction between them. These results demonstrated that lysine acetylation of K76 might affect its interaction with BmNPV-p47 (Fig. 5).



SAMPLE	PLASMID	INTERACTION
1	pGBKT7-53/pGADT7-T	YES
2	pGBKT7-Lam/pGADT7-T	NO
3	pGBKT7-*lef4*-Wt/pGADT7	NO
4	pGBKT7/pGADT7-*p47*	NO
5	pGBKT7-*lef4*-Wt/pGADT7-*p47*	YES
6	pGBKT7-76Q/pGADT7-*p47*	NO
7	pGBKT7-76R/pGADT7-*p47*	NO

Fig. 5

The effects of lysine acetylation of BmNPV-LEF4 K76 on its association with BmNPV-P47

Y2H assay showing the interaction between *lef*-4-Wt, the various mutants and P47. The blue colonies represent an interaction with P47 and those without colonies represent loss interaction. 1. Positive control sample: pGBKT7-53/pGADT7-T; 2. Negative control: pGBKT7-Lam/pGADT7-T; 3. Toxicity test (pGBKT7-LEF4/pGADT7); 4. Auto activation test (pGBKT7/pGADT7-p47); 5. Wild type BmNPV LEF4/ p47 (pGBKT7-LEF4 /pGADT7-p47); 6. Mutant LEF4 K76Q/p47 (pGBKT7-LEF4 K76Q); 7. Mutant LEF4 K76R/p47 (pGBKT7-K76R/ pGADT7-p47).

Discussion

Post-translational modifications are essential for the effectiveness of the viral infection process (Tsai *et al.,* 2020; Ma *et al.*, 2020). Several studies have reported that the post translational modifications of viral proteins influence and regulate gene expressions, protein stability, and cellular localization. Recent studies have also demonstrated that modification of some baculovirus proteins led to the regulation of its infection cycle. For instance, the phosphorylation of P6.9 at Ser/Thr 7 is important for the late viral gene expression and facilitation of viral infectivity (Li *et al.,* 2015; Tsai *et al.*, 2020). Another study revealed that the phosphorylation at serine 92 of P10 helps structural conformational changes during viral infections, enhancing the dissemination and survival of the virus in the host cell (Raza *et al.,* 2017). Additionally, it has been reported that the glycosylation of GP64 affects the formation of budded virus (BV) membrane, the substitution of three histidine residues (H245, H304 and H430) with Alanine was also reported to hinder membrane fusion process, decreasing the conformation fusion pH (Li and Blissard, 2011). Besides, it has been demonstrated that ubiquitination of specific nucleocapsid protein AC66 is required for effective budded virus (BV) production. Moreover, AC141 and AC66 proteins interact with vUbi to form a complex molecule, which is needed for nucleocapsid formation (Leisy and Rohrmann, 2000).

Acetylation is important for the modulation of gene expression in many eukaryotes and prokaryotes (D'Orso and Frankel, 2009; Thao *et al.*, 2010; Kuczynska *et al.*, 2016). However, to date the mechanism and role played by acetylation modification in BmNPV infection is still unclear. Hu *et al.,* 2015 demonstrated that LEF4 of BmNPV can be acetylated at K76 during the viral infection (Hu *et al.,* 2015). In order to investigate whether modification of LEF4 K76 could have effects on late genes, we transfected BmN cells with *lef4*-Wt, K76Q and K76R bacmids and at different times, 24, 48, and 72 hpt, the mRNA level of *vp39* and *p10* was analyzed. We found that at 48 and 72 hpt, cells transfected with K76R showed a relatively lower level of mRNA for both *vp39* and *p10* compared to the cells transfected with *lef4*-Wt and K76Q. Previously, it has been reported that suppression of *lef4* gene in viral genome caused a significant reduction of some late genes, such as *p10*, *vp39*, and *polh* (Passarelli and Miller, 1993; Carstens *et al*.,1994; Knebel-mo *et al.,* 2006). These results corresponded to our findings that deacetylation of K76 caused an alteration of the transcriptional level of *vp39* and *p10* genes upon the viral infection process. Next, we determined the effects of acetylation modification on early genes by analyzing the mRNA level of *lef3*. It was found that both LEF4 K76Q and LEF4 K76R did not in any way affect transcription of *lef3*. Indeed, it supports the finding that LEF4 is not involved in the transcription of early genes (Knebel-Mo *et al.*, 2006). Since it has previously been demonstrated that *lef3* is an early gene essential for viral DNA replication (Yu and Eric, 2010), the results suggest that modification of LEF4 K76 has no effect on DNA replication. This was consistent with the results of viral genome replication (data not shown), which also showed comparable viral DNA copy numbers in BmN cells transfected with *lef4*-Wt, K76Q and K76R. Furthermore, it can also be said that most of the genes involved in the viral replication are either transcribed in very early or delayed early phase, hence could not be influenced by modified *lef4* gene which is associated with regulation of late and very late genes. We further investigated how modified LEF4 may interfere with the viral infection process. The results showed that K76R impaired the viral infection ability while acetylation of K76Q had no effect on the infection and acted as *lef4*-Wt virus. Most of the baculoviral genes that are reported to be associated with structural functions are transcribed in the late or very late phase, VP39 is among them alongside with P6.9, VLF-1, GP64, and P10, which had been found to be required in the production of the nucleocapsid (Wang *et al.*, 2010). The function of the viral capsid is well known as it is involved in the initiation of viral infection mechanism once it comes into contact with the host cells receptor. The present evidence indicates that mutation of the *vp39* gene hampers normal capsid assembly, thus affecting the production of budded virions and the disruption of the viral infection process (Kokusho *et al.*, 2017). Consistent with the study done on the nucleoprotein NP gene of in-

fluenza virus, which is essential for viral RNA replication and transcription, the results showed that deacetylation of K299 also caused a severe reduction of infectious viral particles (Giese *et al.*,2017). Therefore, we speculate that deacetylation of LEF4 at the K76 site caused attenuation of late and very late genes transcription, and these genes include structural genes necessary for the production of budded virus, which could probably be the reason for the slowdown of the viral infection.

Finally, many biomolecular interactions occur inside the cells of a living organism and this forms a complex molecular machine to facilitate specific functions in the cell (De la Rivas and Fontanillo, 2010). A previous report on association between LEF4 and P47 suggested their roles in the transcription of late and very late genes, however little is known about that of the P47 (Crouch *et al.,* 2007). Corresponding to our Y2H results, it was found that LEF4 interacted with P47. Interestingly, there were no observed interactions between the both mutants (K76Q & K76R) and P47, suggesting that lysine acetylation reaction occurred through the addition of active acetyl co-A enzyme group to the BmNPV-LEF4 K76. Following the host N-terminal acetyltransferase (NAT) enzyme, the acetylation mimicking protein K76Q, having lysine residue (positive charge) replaced with glutamine (neutral), might have created a small variation in terms of the hydrophobicity and polarity. On the other hand, the presence of a positive charge on arginine amino residue of the deacetylation mimicking protein K76R suggests a low possibility of its interaction with P47 as a result of low hydrophobicity. We, therefore, speculate that K76 is crucial for the stability of LEF4 interaction.

In summary, this study demonstrates that deacetylation of LEF4 K76 disrupts the transcription of late (*vp39*) and very late gene (*p10*), slowing down the viral infectivity, suggesting that acetylation of LEF4 K76 is important for the LEF4 to properly perform its guanylyl transferase function during the mRNA capping process. However, more research should be done to elucidate the role of acetylation modification on the LEF4.

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