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Overexpression of Bm65 correlates with reduced susceptibility to inactivation by UV light

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ABSTRACT

Ultraviolet (UV) light is one of the factors that causes baculovirus inactivation. However, little is known about the response of baculoviruses to UV light. In the present study, *Bombyx mori* nucleopolyhedrovirus (*Bm*NPV) orf 65 (Bm65), the homolog of *Autographa californica* nucleopolyhedrovirus orf 79 (Ac79), a predicted endonuclease, was analyzed. Preliminary results indicated that Bm65 mainly accumulated within the nucleus and could improve the survival rate of *Escherichia coli* (*E. coli*) and *Bm*NPV BVs after UV radiation, suggesting that Bm65 was involved in the repair of UV-induced DNA damage.

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1. Introduction

Baculoviruses are enveloped double-stranded DNA viruses that primarily infect insects from the Lepidoptera, Hymenoptera and Diptera orders. At present, baculoviruses can be safely used as long-term and environmentally friendly biopesticides. However, UV radiation limits their wide application. Previous studies showed that baculoviruses lose most of their viability within 24 h when exposed to sunlight (Black et al., 1997; David et al., 1967; Petrik et al., 2003).

The key target molecule of UV radiation is DNA. Cyclobutane pyrimidine dimers (CPDs) and pyrimidine 6–4 pyrimidone photoproducts (6–4 PPs) are the main photolesions in genomic DNA after UV light irradiation (Chiganças et al., 2004). Almost all organisms have evolved repair mechanisms for UV-induced DNA lesions. There are three pathways to repair pyrimidine dimers, including photoreactivation, light-independent nucleotide excision repair (NER) and base excision repair (Xu et al., 2010). Photoreactivation is the photolyase-mediated light-dependent repair of UV-induced damage. Nucleotide excision repair widely exists in prokaryotic and eukaryotic organisms and requires a series of enzymes, including DNA endonuclease, DNA polymerase, ligase, and so on. In addition, some specific organisms, including viruses, also use base excision repair. Glycosylase is the key enzyme in base excision repair. In sequenced baculoviruses, the photolyase gene was only found in *Trichoplusia ni* single nucleocapsid nucleopolyhedrovirus (*Tn*SNPV) and *Chrysodeixis chalcites* nucleopolyhedrovirus (*Chch*NPV) (Van Oers et al., 2005, 2004; Biernat et al., 2012; Willis et al., 2005). However, it is unclear whether UV-induced damage repair mechanisms also exist in other baculoviruses.

*Bm*NPV is a major pathogen of silkworm and causes serious economic loss in silk production. It is still unknown whether *Bm*NPV possesses the ability to repair UV-induced damage. Our previous study showed that Bm65, the homolog of Ac79, was an early gene and was not associated with viral DNA replication (Tang et al., 2013). In previous study, Bm65 was found to be a member of the GlyIleTyr–TyrIleGly (GIY–YIG) nuclease superfamily and was expressed in *Escherichia coli* in a soluble form, which was used to investigate the effect of Bm65 on the survival rate of *E. coli* after UV treatment. Additionally, overexpression of Bm65 was mediated by recombinant viruses in BmN cells that were exploited to study the effect of Bm65 on the viability of *Bm*NPV budded viruses (BVs) after UV treatment. These results indicated that Bm65 effectively reduced the susceptibility of *E. coli* and *Bm*NPV BVs to UV light inactivation in the dark. It was inferred that Bm65 was involved in the dark repair of UV-induced DNA damage.

2. Materials and methods

2.1. Bacmid, bacterial strains, virus, and cells Bm

NPV bacmid was donated by Dr. Enoch Y. Park (Department of Applied Biological Chemistry, Shizuoka University, Japan) and was propagated in *E. coli* strain DH10B cells containing the pMON7124 helper plasmid. The *E. coli* BL21 strain and pUC18

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plasmid were maintained in our laboratory. The pFB-P_{ie-1}-egfp plasmid containing the *egfp* gene (promoter from *Bm*NPV *ie*-1) was constructed as previously described and was used for constructing the recombinant *Bm*NPV overexpressing Bm65 (Shen et al., 2009). The pHTB-egfp plasmid was constructed by Dr. Li in our laboratory and was used for constructing the recombinant *Bm*NPV expressing the Bm65-EGFP fusion protein. BmN cells were cultured at 27 °C in TC-100 insect medium supplemented with 10% fetal calf serum (Gibco).

2.2. Construction of recombinant BmNPV expressing the Bm65-EGFP fusion protein

pFastHTB-P_{Bm65}-Bm65-egfp was constructed as follows. Briefly, *egfp* was amplified by PCR using primers *egfp*-F and *egfp*-R. Purified PCR product was ligated into pFastHTB to generate recombinant plasmid pHTB-egfp. Then, a DNA fragment containing the Bm65 gene with a native promoter was amplified by PCR using primers Bm65-F2 and Bm65-R2. After digestion with *Sna* B I/*Pst* I, the purified fragment was ligated into pFastHTB-egfp to generate the final recombinant plasmid pFastHTB-P_{Bm65}-Bm65-egfp. DH10B competent cells containing the pMON7124 helper plasmid and *Bm*NPV bacmid were transformed with the pFastHTB-P_{Bm65}-Bm65-egfp. The cassette of P_{Bm65}-Bm65-egfp was transposed into the mini-attTn7 locus of the *Bm*NPV bacmid to generate the resulting recombinant bacmid $pBm^{PBm65\text{-}Bm65\text{-}egfp}$ (Fig. 2A). $pBm^{PBm65\text{-}Bm65\text{-}egfp}$ (2 μg/well) was extracted from DH10B cells and transfected into BmN cells for preparing recombinant BVs ($vBm^{PBm65\text{-}Bm65\text{-}egfp}$) using Cellfectin Reagent (Invitrogen Life Technology). The supernatant containing $vBm^{PBm65\text{-}Bm65\text{-}egfp}$ was harvested at 72 h p.t. (hours post transfection). The primers used in the current study are listed in Table 1.

2.3. Subcellular localization of Bm65-EGFP in infected cells

To examine the subcellular localization of Bm65, 1×10^3 BmN cells were plated in a 60-mm dish, which was infected with $vBm^{PBm65\text{-}Bm65\text{-}egfp}$ for the expression of Bm65-EGFP fusion proteins. Briefly, BmN cells infected with recombinant $vBm^{PBm65\text{-}Bm65\text{-}egfp}$ were cultured at 27 °C for 6, 12, 24 and 48 h, respectively. Then, the cells were washed with PBS, fixed with 4% paraformaldehyde for 15 min, washed three times with PBS for 10 min, and permeabilized in 0.1% Triton X-100 for 15 min. After washing with PBS three times, the cells were stained with DAPI (Sigma, USA) for 10 min, washed three times with PBS, and then were directly observed and photographed using Confocal Laser Scanning Microscopy (Leica, Germany). Fluorescence emission was excited at 488 and 340 nm to detect the presence of EGFP and DAPI, respectively.

2.4. Soluble Bm65 expressed in BL21

To determine the expression of Bm65 in BL21, pET30a-Bm65 was constructed. The coding region of Bm65 was amplified using primers Bm65-F1 and Bm65-R1 from *Bm*NPV genomic DNA. The amplified fragment was cloned into expression vector pET30a (Novagen, USA) to generate pET30a-Bm65; pET30a-Bm65 was later transformed into BL21 cells to express Bm65. Briefly, BL21 cells harboring pET30a-Bm65 were cultured in LB culture medium at 37 °C. When the culture reached an OD600 value of 0.5, isopropyl-β-D-thiogalactopyranoside (IPTG) was added at a final concentration of 0.8 mM to induce the expression of the 6× His-Bm65 fusion protein. After shaking for 12 h, the cells were harvested from LB medium by centrifugation at 7,000*g* for 10 min at 4 °C and were then washed once with PBS. Then, the cell pellet was suspended in PBS (pH 7.4) and disrupted by sonication at 180 Hz. The lysate was centrifuged at 10,000*g* for 30 min at 4 °C. The supernatant was harvested and subjected to 15% SDS–PAGE analysis to separate soluble 6× His-Bm65. Then, the target protein was transferred onto a polyvinyl difluorene (PVDF) membrane (Millipore cat. No. IPVH00010). The blots were blocked with 5% nonfat milk in PBS for 1 h and then incubated with monoclonal antibodies (Abcam) against 6× His-tag. Goat anti-mouse IgG conjugated to horseradish (Abmart) was used as the secondary antibody. The hybridization signal was visualized by using a DAB kit.

Table 1
Primers used in the study.

Primer name	Primer sequence (5′–3′)	Enzyme digestion sites
Bm65-F1	AT<u>GGATCC</u>ATGGCGACGACTCT	*Bam*H I
Bm65-R1	GC<u>AAGCTT</u>CAACTTATTTGCTAACAGA	*Hind* III
P_{ie-1}-F	CG<u>AAGCTT</u>CGTCGAACATGCCTATTTAC	*Hind* III
P_{ie-1}-R	TA<u>CTGCAG</u>AGTCACTTGGTTGTTCACGAT	Pst I
*Bm*65-F2	AG<u>TACGTA</u>ATGGCGACGACTCTGTACA	Sna B I
*Bm*65-R2	TA<u>CTGCAG</u>CAACTTATTTGCTAACAGAAATTT	Pst I
egfp-F	TA<u>CTGCAG</u>ATGGTGAGCAAGGGCGAGGA	Pst I
egfp-R	TA<u>CTCGAG</u>TTACTTGTACAGCTCGTCCATGCCG	Xho I
Bm65-F3	TA<u>CTGCAG</u>ATGGCGACGACTCTGTAC	Pst I
Bm65-R3	TA<u>GGATCC</u>TTACAACTTATTTGCTAACAGAA	*Bam*H I

Note: underlined letters indicate restriction enzyme digestion sites.

2.5. Effects of Bm65 on the UV sensitivity of E. coli

Considering that both light repair and nucleotide excision repair exist in *E. coli*, *E. coli* cells harboring pET30a-Bm65 treated with UV were cultured in a light incubator or in the dark to study whether Bm65 functioned in the light or in the dark. Briefly, the host strain BL21 cells containing pET30a-Bm65 or pET30a were cultured in LB medium at 37 °C. When the culture reached an OD_{600} of 0.6, IPTG was added to the LB medium (at a final concentration of 0.8 mmol/L IPTG), and these cells were further cultured for 5 h. Then, these cells were counted using a blood cell counting chamber and were diluted with LB culture medium at the same concentration. The two bacterial cultures were spread onto LB plates containing 50 μg/mL kanamycin and were exposed to 0.05 J/m^2, 0.15 J/m^2, 0.3 J/m^2 or 0.5 J/m^2 UV (254 nm wavelength) light generated by a hand-held wand. The first group treated with UV radiation was cultured in a 37 °C light incubator, the second group that was treated with UV radiation was wrapped in foil (dark culture) and cultured in a 37 °C incubator, and the third group that was untreated with UV radiation was cultured in a 37 °C incubator under routine conditions as controls. After incubation for 18 h, the colonies were counted. The ratio of the bacteria colonies of the experimental group to that of the control group was used as the survival rate. Each sample was assayed at least in triplicate. The above experiments were performed three times. Statistical analysis was performed using single factor analysis of variance.

2.6. Construction of recombinant BmNPV overexpressing Bm65

To conveniently evaluate the effect of UV radiation on viral propagation, the expression cassette of P_{ie-1}-*egfp* was inserted into the mini-attTn7 locus of *Bm*NPV bacmid by site-specific transposition. The construction of P_{ie-1}-*egfp* was as follows. Briefly, a DNA fragment of the *ie*-1 promoter was amplified from the *Bm*NPV genome using primers P_{ie-1}-F and P_{ie-1}-R. After digestion with *Hind* III/

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