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BmNPV TURNS HOST AUTOPHAGY TO ITS ADVANTAGE: WHAT THE EVIDENCE SHOWS

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Abstract: Bombyx mori nucleopolyhedrovirus (BmNPV) is one of the most harmful pathogens in sericulture, causing serious losses in cocoon production. Recent research has revealed that BmNPV can induce autophagy in host cells during the early stage of infection. In infected BmN-SWU1 cells, the formation of autophagosomes and lipidated ATG8 proteins increases significantly, together with the upregulation of several autophagy-related genes, including Atg7, Atg9, Atg8, and Atg12. Silencing these genes reduces the expression of viral genes such as ie-1, vp39, and p10, indicating that the virus benefits from the host's autophagy system. This review summarizes the molecular evidence showing how BmNPV utilizes host autophagy to enhance its replication and discusses its potential implications for virus control and silkworm breeding strategies.

Review article: Bombyx mori Nuclear Polyhedrosis Virus (BmNPV) Induces Host Cell Autophagy to Benefit Infection

Baculoviruses are a family of DNA viruses that have a large, circular, supercoiled, and double-stranded DNA-containing genome that infect insects, particularly of the order Lepidoptera. Bombyx mori nucleopolyhedrovirus (BmNPV), one of the best characterized baculoviruses, has two virion phenotypes during its infectious life cycle: occlusion-derived virus (ODV) and budded virus (BV).[1]

Introduction

Silkworm (Bombyx mori) is an economically important insect that supports the silk industry worldwide. However, its productivity is often threatened by viral diseases, among which Bombyx mori nucleopolyhedrovirus (BmNPV) is the most destructive. This virus belongs to the Baculoviridae family and causes nuclear polyhedrosis, a disease that leads to high larval mortality and serious economic losses in sericulture.

In recent years, research on the interaction between BmNPV and host defense mechanisms has advanced rapidly. One of the most intriguing discoveries is that the virus can interfere with the autophagy pathway, a conserved cellular process responsible for the degradation of damaged proteins and organelles. Autophagy generally protects cells against stress and infection, but several viruses have evolved to exploit this process to enhance their own replication.

Studies using silkworm ovarian cell lines (BmN-SWU1) revealed that BmNPV infection triggers typical signs of autophagy, such as the formation of double-membrane autophagosomes, accumulation of lipidated ATG8, and the upregulation of autophagy-related genes (Atg7, Atg9, Atg8, Atg12). Interestingly, silencing these genes reduces viral gene expression, suggesting that autophagy benefits the virus rather than inhibiting it.

This chapter provides an overview of the molecular relationship between BmNPV infection and host autophagy, highlighting how the virus manipulates this cellular mechanism to promote its own survival. Understanding this interaction is essential for developing new molecular strategies for virus control and resistant silkworm breeding.

[1] Blissard G.W., Rohrmann G.F. Baculovirus diversity and molecular biology. Annu. Rev. Entomol. 1990;35:127–155. doi: 10.1146/annurev.en.35.010190.001015. [DOI] [PubMed] [Google Scholar]

Chapter

2.1 Materials and methods: Cells, viruses, and plasmids

In studies focused on the interaction between Bombyx mori nucleopolyhedrovirus (BmNPV) and host cell autophagy, the BmN-SWU1 cell line is considered one of the most reliable experimental systems. This cell line, derived from B. mori ovarian tissue, provides an ideal in vitro model for observing viral infection processes and gene expression changes under controlled conditions. From my understanding, maintaining these cells at 27 °C in TC-100 insect medium with 10% fetal bovine serum (FBS) ensures optimal physiological conditions similar to those in living silkworm tissues. The use of penicillin and streptomycin in the culture is also essential to prevent any bacterial contamination that could interfere with virus–host interactions.

In this work, the virus strain (BmNPV, GenBank No. NC001962.1) was propagated in the same BmN-SWU1 cells, which is quite logical since it maintains viral adaptation and stability. This consistent culture system allows the researchers to minimize external variables and focus on the molecular mechanisms of infection.

What I found particularly interesting is their use of various plasmid constructs to manipulate autophagy-related genes (Atgs). They prepared both overexpression and CRISPR/Cas9 knockout plasmids for genes such as Atg3, Atg4, Atg5, Atg7, Atg9, and Atg12. This dual approach—enhancing or silencing specific Atgs—makes it possible to compare how each gene contributes to the autophagic response and viral replication efficiency.

For visual observation, fusion plasmids encoding BmAtg8 tagged with fluorescent proteins (EGFP or EGFP-RFP) were used. I think this is an elegant choice because these fusion markers allow real-time visualization of autophagosome formation and autophagic flux through confocal microscopy. It's a practical way to distinguish between early and late stages of autophagy.

In summary, the methodology presented here combines cell culture, molecular cloning, and CRISPR-based editing in a very systematic way. It provides a clear model to study how BmNPV manipulates host autophagy at both cellular and genetic levels, and I believe such an approach can serve as a strong reference framework for future research on silkworm–virus interactions.

2.2. Discovery of Virus infection and plasmid transfection

When I studied this part of the paper, I found it especially interesting how carefully the authors designed both the infection and transfection steps to explore the relationship between BmNPV and host autophagy. They used Bombyx mori ovarian cells (BmN-SWU1 line), which were grown as monolayer cultures — a standard and stable system that allows uniform exposure of cells to the virus. Before infection, the cells were seeded into six- and twenty-four-well plates, which helps control experimental conditions and ensures reproducibility.

The infection process itself was carried out using a multiplicity of infection (MOI) of 1, meaning that, on average, one virus particle infects each cell. I think this is a smart choice — it's enough to ensure almost every cell gets infected, but not too high to cause stress or excessive cell death. The cells were allowed to absorb the virus for about two hours, after which the unbound viral particles were washed off with PBS. I find this washing step particularly important — it makes sure that only the truly infected cells remain, which leads to cleaner results and more accurate analysis of viral behavior.

After infection, the researchers performed plasmid transfection to alter the expression of specific autophagy-related genes (Atg3, Atg4, Atg5, Atg7, Atg9, Atg12, etc.). They used liposome-based reagents, which is a common and gentle method for introducing DNA into insect cells. Interestingly, they optimized the plasmid concentration for each plate type — using 1.5 μg for six-well plates and 0.5 μg for twenty-four-well plates — showing how much attention they gave to maintaining balance between efficiency and cell health.

What caught my attention most was how they combined viral infection and autophagy observation within the same system. Using fluorescent plasmids tagged with EGFP and RFP on Atg8, they could actually see autophagosomes forming inside infected cells under the confocal microscope. This dual-fluorescent strategy gave them both visual and quantitative insight into how the virus affects autophagic activity.

This experiment beautifully demonstrates how molecular biology and cell imaging can work hand in hand. It not only revealed that autophagy is triggered early after BmNPV infection but also hinted that changes in Atg gene expression directly shape how efficiently the virus replicates. I found this integration of infection, genetic manipulation, and live-cell imaging particularly impressive — it shows a clear, well-controlled approach to studying virus–host interactions at a cellular level.

2.3 Discovery of Real-Time PCR — gene expression analysis of autophagy and viral genes

Among all the experimental parts of this study, the Real-Time PCR analysis particularly caught my attention. It is through this method that the authors managed to connect Bombyx mori nucleopolyhedrovirus (BmNPV) infection with changes in the host's autophagy-related gene expression. I found it fascinating how this quantitative approach revealed not only which genes respond to infection, but also how their regulation can influence viral replication itself.

In this experiment, total RNA was extracted from BmN-SWU1 cells under several carefully designed conditions — uninfected controls, BmNPV-infected cells, and cells that had been transfected with either Atg overexpression or knockout plasmids prior to infection. I think this comparative design is very logical because it allows for observing how the manipulation of Atg genes affects both the host defense system and the virus's ability to replicate.

After RNA isolation, the researchers used the SYBR Green-based qPCR system (Bio-Rad CFX Connect) to measure transcription levels. The thermal cycling program was typical for precise amplification — 95 °C denaturation followed by 44 amplification cycles. What impressed me is their use of BmRPL3 as an internal control gene, which ensures reliable normalization between samples. Such attention to detail strengthens the credibility of their quantitative data.

The primer set they designed was quite comprehensive — covering nearly all the major autophagy-related genes (Atg1–Atg18) as well as key viral genes such as ie-1, vp39, and p10. This wide coverage makes it possible to trace dynamic changes across different infection stages. From their results, it was clear that many Atg genes, especially Atg7 and Atg9, were highly upregulated during the early infection phase. Since these genes are known to regulate autophagosome formation and membrane trafficking, this finding makes perfect sense biologically — it suggests that the virus stimulates host autophagy right from the beginning of infection.

What I found most intriguing is how silencing Atg7 and Atg9 through the CRISPR/Cas9 system caused a dramatic reduction in viral gene expression, including ie-1, vp39, and p10. This result directly demonstrates that BmNPV depends on the host autophagy machinery for its own

replication. It's a clear example of how a virus can hijack the host's cellular processes to its advantage.
Overall, I think this part of the study is very insightful. The Real-Time PCR data provide convincing molecular evidence that BmNPV not only triggers autophagy but also exploits it to promote efficient infection. From my perspective, Atg7 and Atg9 could serve as important molecular indicators for understanding virus–host interactions and may even become potential targets for antiviral strategies in sericulture research.

2.4 Discovery of knockout efficiency analysis

When I reviewed this part of the experiment, I found it particularly important because it verified whether the CRISPR/Cas9 system truly worked as intended. The authors didn't just assume that the targeted Atg genes were knocked out—they carefully confirmed it through molecular validation. I appreciated this level of precision, as gene-editing experiments often produce mixed populations of cells, and without verification, the results could easily be misleading.
In this section, genomic DNA was extracted from the treated BmN-SWU1 cells using the Wizard Genomic DNA Extraction Kit (Promega, USA). This method provided high-purity DNA suitable for subsequent PCR and sequencing. Specific fragments containing the CRISPR target sites were amplified using gene-specific primers, which allowed direct analysis of the edited regions. These PCR fragments were then inserted into the pMD19-T vector, and sequencing was performed using universal M13 primers. I think this cloning step was a smart choice—it allowed them to precisely check whether any insertions, deletions, or point mutations had occurred at the sgRNA target sites. By comparing the sequencing data with the reference genome, they could clearly see which cells had undergone successful genome editing. To me, the most valuable aspect of this analysis is that it goes beyond technical verification—it ensures the biological accuracy of the subsequent experiments. Confirming that the Atg3, Atg4, Atg5, Atg7, Atg9, and Atg12 genes were successfully edited means that any changes observed in viral gene expression or autophagy activity were genuinely the result of those specific knockouts, not experimental noise.
In short, this verification step added a solid layer of credibility to the entire study. It provided the assurance that the observed relationship between autophagy and BmNPV infection was based on true genetic manipulation rather than assumption. Personally, I think this careful confirmation of knockout efficiency represents the hallmark of good molecular biology practice—thorough, reproducible, and scientifically trustworthy.

2.5. Discovery of statistical analysis

When I read this section, I immediately noticed how systematically the authors treated their data — not just presenting results, but ensuring every finding was statistically meaningful. They used GraphPad Prism 5.0**,** a software I'm also familiar with, which is highly reliable for analyzing biological experiments and visualizing quantitative trends. What I liked most is that every experiment was performed at least three times, with results expressed as mean ± standard deviation (SD). This repetition makes the data much more convincing and shows that the observations were not one-time events but reproducible biological phenomena.
To compare differences between control and treatment groups, they applied the Student's t-test, which is the standard method for identifying whether observed differences are statistically significant. A p-value below 0.05 was considered significant, and $p < 0.01$ represented a highly significant result. These statistical thresholds are small details, but they make a big difference —

they help separate genuine biological effects from random variation. What I found particularly meaningful was how these statistics were used to support specific experimental findings — for instance, the significant upregulation of Atg7 and Atg9 after BmNPV infection, or the sharp decrease in viral gene expression following Atg knockout. The use of consistent statistical criteria across all experiments strengthens the overall conclusion that BmNPV-induced autophagy is not a coincidence but an active mechanism that benefits the virus.

Chapter

Results

Discovery of BmNPV infection induces autophagy in BmN-SWU1 cells

When I analyzed this part of the study, it immediately caught my attention how Bombyx mori nucleopolyhedrovirus (BmNPV) interacts with the host cell's internal defense system. What fascinated me most was that instead of being suppressed by the cell's natural mechanism, the virus actually activated the host autophagy pathway—a process that normally protects cells under stress.

To visualize this, the researchers used three complementary approaches: transmission electron microscopy (TEM), fluorescent ATG8-GFP tracking, and Western blot analysis. Each method provided a unique layer of evidence, but together they painted a very clear picture of what happens inside the infected cell.

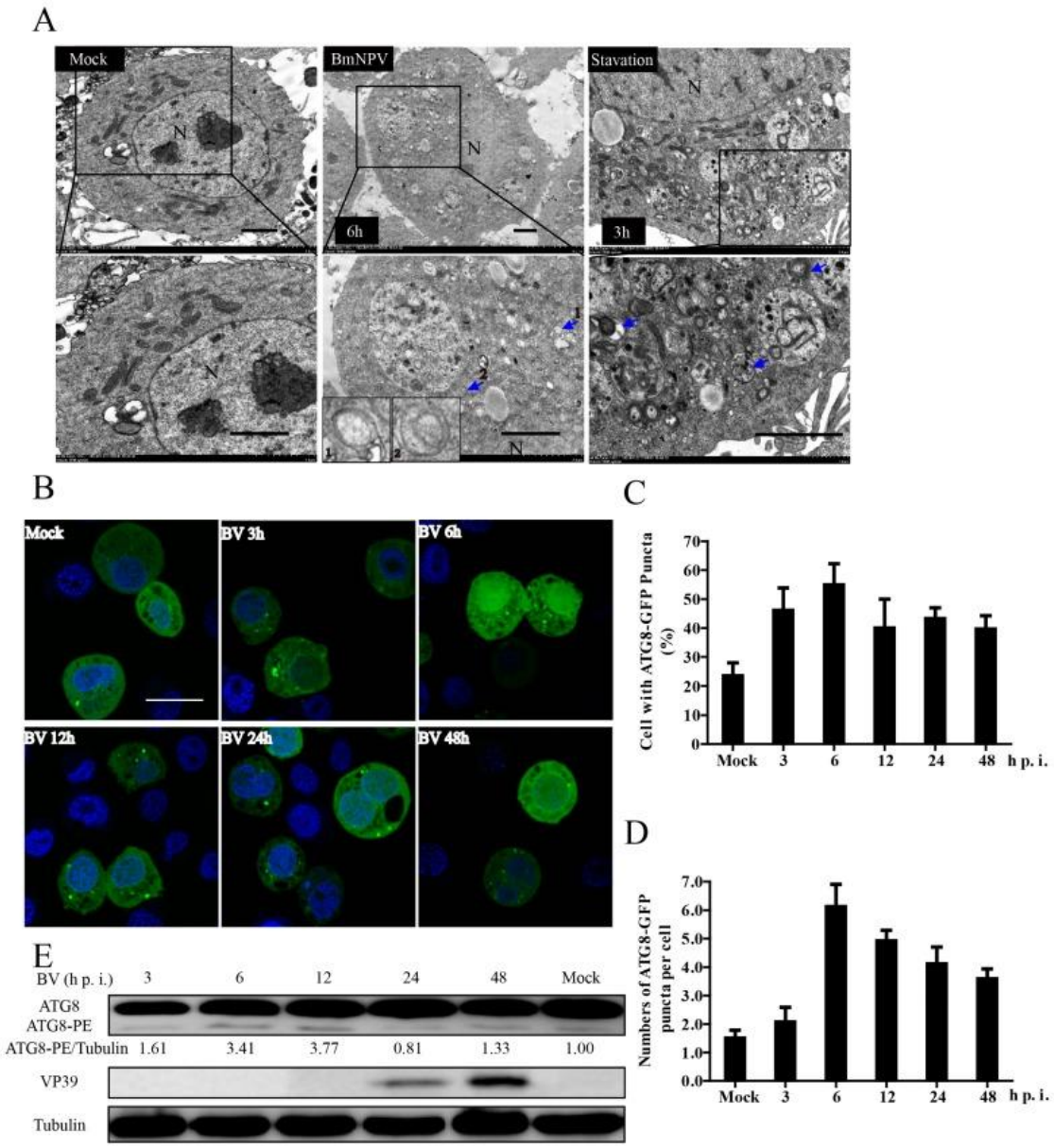
In the TEM images (Figure 1A), I found it remarkable that the uninfected control (mock) cells showed normal cytoplasmic structures with no autophagic vesicles. However, within just six hours after infection, the cytoplasm of BmNPV-infected cells was filled with numerous double-membrane vesicles—classic hallmarks of autophagosomes. To make sure this was not just a random observation, they compared it with starvation-induced cells, which are known to trigger autophagy. Seeing a similar pattern there confirmed that the virus indeed stimulates an autophagy-like response. The fluorescent ATG8-EGFP experiment (Figure 1B) further strengthened this finding. I found this part especially interesting because ATG8 (the insect equivalent of LC3 in mammals) lights up as bright green puncta when autophagosomes form. In BmNPV-infected cells, the number of these fluorescent spots significantly increased within 3–6 hours after infection. The statistical data (Figures 1C and 1D) showed that the proportion of cells with ATG8-GFP puncta rose from less than 25% in the control to over 50% at 6 hours post-infection. The number of puncta per cell also nearly doubled, clearly indicating an early burst of autophagic activity.

However, I found it intriguing that after 24–48 hours, the number of puncta began to decrease again. This suggests that autophagosomes might have been rapidly degraded, or perhaps the autophagic flux had accelerated to balance the process. It's like the cell and the virus were continuously adjusting their battle inside the cytoplasm.

Finally, the biochemical analysis (Figure 1E) added strong molecular support to the visual data. The conversion of ATG8 to its lipidated form, ATG8-PE (also known as ATG8-II), increased dramatically—peaking at 12 hours post-infection with a ratio of 3.77 relative to tubulin. To me, this was the most convincing piece of evidence that autophagy was not only triggered but also maintained in an active state during the early infection phase. At the same time, the gradual appearance of the viral protein VP39 confirmed that infection was progressing successfully.

Altogether, this combination of morphological, fluorescent, and molecular evidence made it clear that BmNPV induces a robust autophagic response in BmN-SWU1 cells soon after infection. In my interpretation, this shows that the virus cleverly utilizes the host's own survival

machinery to create a more favorable environment for its replication. I found this mechanism both surprising and elegant—it reveals how a pathogen can turn a defensive cellular process into a tool for its own advantage.



This figure was taken from the results section of the article **"Bombyx mori Nuclear Polyhedrosis Virus (BmNPV) induces host cell autophagy to benefit infection"**[2]

Related Terms: While studying the interaction between Bombyx mori nucleopolyhedrovirus (BmNPV) and its host, I came across several key biological terms that are closely related to this

[2] https://www.ncbi.nlm.nih.gov/core/lw/2.0/html/tileshop_pmc/tileshop_pmc_inline.html?title=Click%20on%20image%20to%20zoom&p=PMC3&id=5795427_viruses-10-00014-g001.jpg].

research and help explain the mechanisms involved. Understanding these terms is essential to fully grasp how the virus manipulates host cellular machinery.

Autophagy: Autophagy is a conserved cellular process through which cells degrade and recycle damaged organelles and proteins. In insects like Bombyx mori, autophagy plays a dual role — it maintains cellular balance under stress, but it can also be hijacked by viruses for their benefit. I found it fascinating that in the case of BmNPV infection, autophagy does not simply serve as a defense response; rather, the virus actively stimulates it to support its replication.
ATG Genes (Autophagy-Related Genes): These genes are at the core of the autophagy pathway. In the study, genes such as Atg3, Atg5, Atg7, Atg9, and Atg12 were shown to be particularly important. Their products are directly involved in forming autophagosomes — the double-membrane vesicles that capture cytoplasmic material for degradation. I was especially intrigued by Atg7 and Atg9, which appear to have crucial roles during the early stages of BmNPV infection.
Autophagosomes and autophagic flux: An autophagosome is a vesicle that engulfs cytoplasmic components before fusing with lysosomes for degradation. The term autophagic flux refers to the entire process — from formation to degradation of these vesicles. In this study, both TEM images and fluorescent ATG8-EGFP markers revealed that BmNPV infection enhances autophagosome formation early on, followed by increased autophagic flux as the infection progresses. I see this as a dynamic balance — the cell tries to survive, while the virus adapts to exploit that very response.
CRISPR/Cas9 Knockout: This genome-editing technology was used to selectively disrupt specific Atg genes, helping to reveal their individual roles in autophagy and viral replication. For me, this part of the study was particularly enlightening, because it proved that when Atg7 or Atg9 were knocked out, the replication of BmNPV sharply decreased. This demonstrates a direct dependency of the virus on the autophagy machinery.
Viral Genes (ie-1, vp39, p10): These genes represent different phases of viral replication — ie-1 is expressed early, while vp39 and p10 are structural proteins produced later. Monitoring their expression alongside the host Atg genes provided a clear timeline of how autophagy correlates with viral replication.
In summary, these related terms form an interconnected framework: BmNPV triggers autophagy, activates Atg genes, enhances autophagosome formation, and relies on this process to efficiently express its viral genes. I found this concept very interesting — it shows that even a destructive process like viral infection can use the cell's self-protection system as a supportive mechanism for its own survival.