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Electron Microscopic Visualization of *Dendrolimus spectabilis* Midgut Cells infected by *D. spectabilis* Nuclear Polyhedrosis Virus

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Dendrolimus spectabilis Nuclear Polyhedrosis Virus에 감염된 솔나방유충 중장세포의 전자현미경적 관찰

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Abstract

Dendrolimus spectabilis nuclear polyhedrosis virus(DsNPV)에 감염되어 죽은 솔나방(*D. spectabilis*)유충의 중장세포를 전자현미경(TEM)으로 관찰하였다. 감염된 중장세포의 핵에서 DsNPV는 복제하였으며, DsNPV의 virogentic stroma가 핵속에 나타났고, nucleocapsid도 형성되었다. 또한 NPV의 polyhedra형성을 핵에서 관찰했다.

Introduction

Nuclear polyhedrosis virus (NPV) multiplies *in vivo* and *in vivo* in the nucleus of the infected host cells and produces virions and nuclear polyhedral inclusion bodies(Granados and Lawler, 1981 ; Kundson and Harrap, 1976). The mechanism and pathways of infection for nuclear polyhedrosis virus have been studied in various insects and insect cell lines(Knudson and Harrap, 1976 ; Tanada and Hess, 1976). Several mechanisms which baculoviruses attach and enter into midgut cells of insect larvae *in vivo* have been reported including fusion of the viral envelope and microvilli membrane, viropex, and transport through the intercellular membrane of adjacent cells(Paschke

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and Summers, 1975). Nuclear polyhedral inclusion bodies ingested by insect larvae are dissolved by proteolytic activity of digestive juice in the intestinal lumen(Pritchett *et al.*, 1981) and virus particles are released into the lumen(Pritchett *et al.*, 1982). *Dendrolimus spectabilis* is distributed in Korea and damages lots of pine tree leaves. Therefore the pathogenecity of *D. spectabilis* larvae by *D. spectabilis* NPV(DsNPV) infection in necessary to control of the larvae and to elucidate the infection pathways of the virus.

In this paper the pathogenecity of *D. spectabilis* midgut cells infected by DsNPV were observed.

Materials and Methods

Virus and insect

Dendrolimus spectabilis nuclear polyhedrosis virus(DsNPV) and *D. spectabilis* larvae were used and obtained from the Jungnung Microbiological laboratory, Department of Forest, Seoul.

Viral infection and isolation of the midgut of larvae

D. spectabilis larvae were infected *per os* with DsNPV polyhedra and incubated for 7days, and then the dead larvae were used for isolation of the midgut. The abdominal region of the larvae was sectioned out under a dissecting microscope(Olympus), from which midgut region was extracted and washed in phosphate saline buffer(Lee and Miller, 1978).

Electron microscopy

The extracted midgut region was fixed in cold 2% glutaraldehyde and then in 2.5% paraformaldehyde solution. The fixation was processed standard procedures for observations and visualized with a transmission electron microscope(Zeiss EM 109) at 50 kV.

Results and Discussion

Dendrolimus spectabilis is widely distributed in Korea, Japan and Siberia, which damages lots of pine tree. *D. spectabilis* larvae and nuclear polyhedrosis virus were isolated in Yongin mountin area. From the dead larvae the polyhedral inclusion bodies were purified and then reinfected to the *D. spectabilis* larvae. The reinfected larvae were sectioned for isolation of the infected midgut. The midgut, especially epithelial cells and villi, was observed to examine and cytotoxicity by a transmission electron microscope. *D. spectabilis* nuclear polyhedrosis virus infected in the epithelial cells and replicated slowly. Virogenic stroma of *D. spectabilis* nuclear polyhedrosis virus appeared in the nucleus (Fig. 1), and nucleocapsids were formed from the virogenic stroms(Fig. 2). Also polyhedral inclusion body formation was observable(Fig. 3). Granados(1978) reported that the enlargement of nuclei by *Autographa californica* nuclear polyhedrosis virus and formation of virogenic stroma in the nucleus were observed at 8h postinfection(p. i.) and by 16h p. i. mature virus progeny were observed in the nucleus, cytoplasm, and basal lamina of columnar cells of *Autographa californica* larvae infected with *A. californica* nuclear polyhedrosis virus. Hess and Falcon(1981) observed that *A. californica* nuclear polyhedrosis virus infected, replicated and damaged the midgut of salt marsh caterpillar, *Estigmene acrea*. Also Lee *et al.*(1988) observed the infection

pathway of *Lymantria dispar* NPV in *L. dispar* larvae. The multiplication pattern of the *D. spectabilis* nuclear polyhedrosis virus in the *D. spectabilis* larvae by our observations was similar to the reports.

Summary

Midgut cells of the dead *Dendrolimus spectabilis* larvae by infection of *D. spectabilis* nuclear polyhedrosis virus(DsNPV) were observed by transmission electron microscopy. DsNPV replicated in the nucleus of the infected midgut cells and the virogenic stroma of DsNPV appeared in the nucleus, from which nucleocapsids were formed. Also the formation of polyhedral inclusion bodies were observed in the nucleus.

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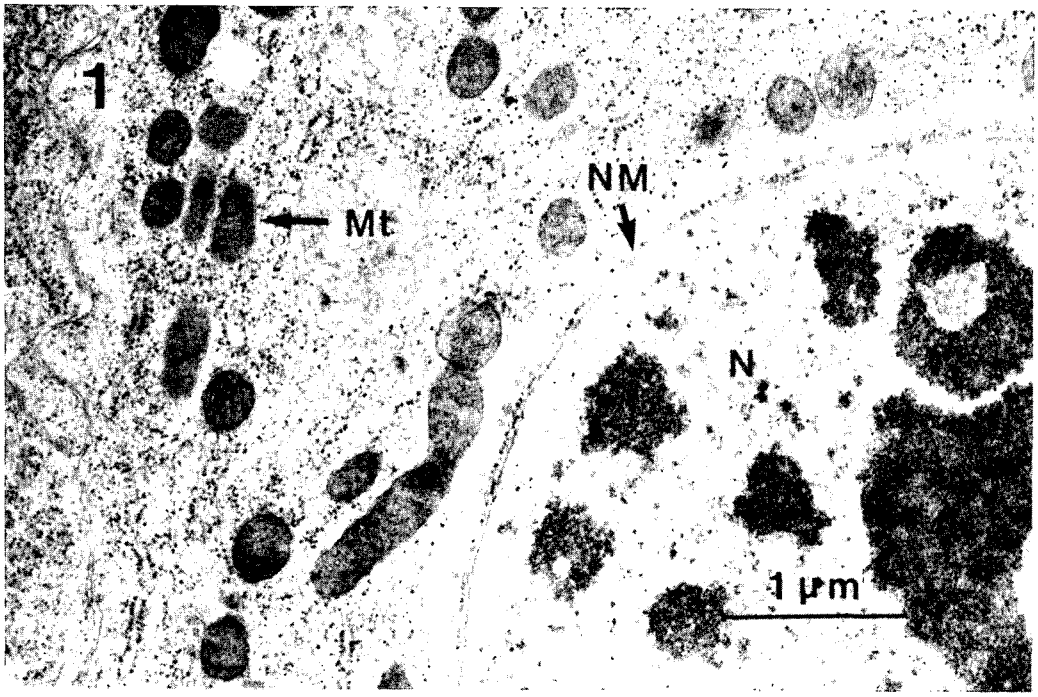
Figure Legends

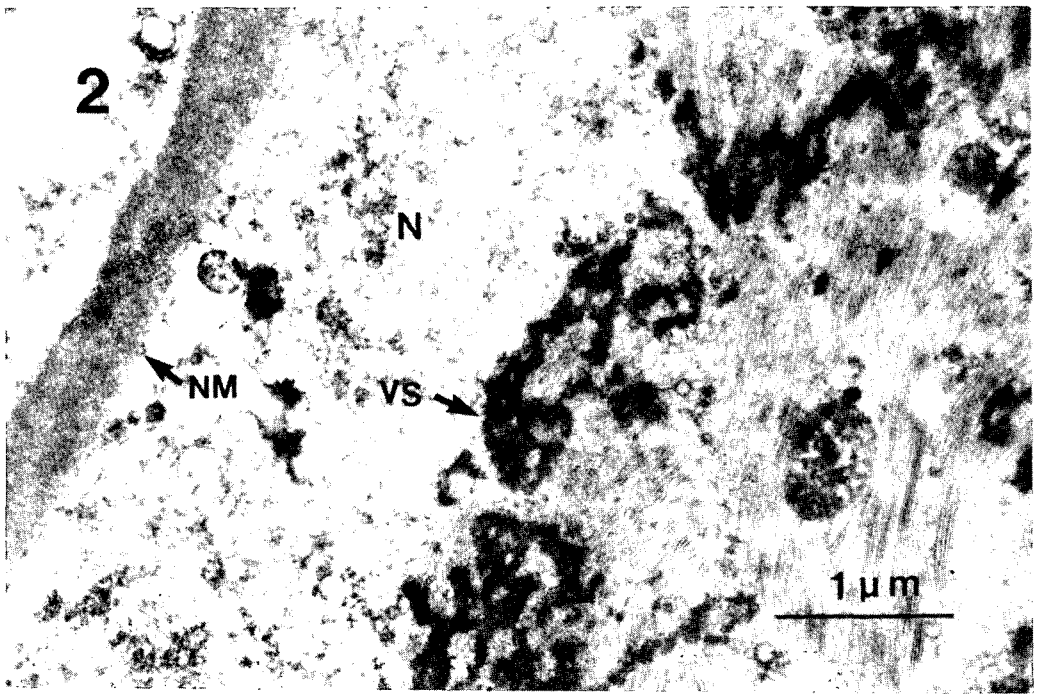
Fig. 1. Electron micrograph of the normal midgut cell of *D. spectabilis* larvae. Symbols : NM, nuclear membrane ; C, cytoplasm ; CM, cell membrane ; Mt, Mitochondria

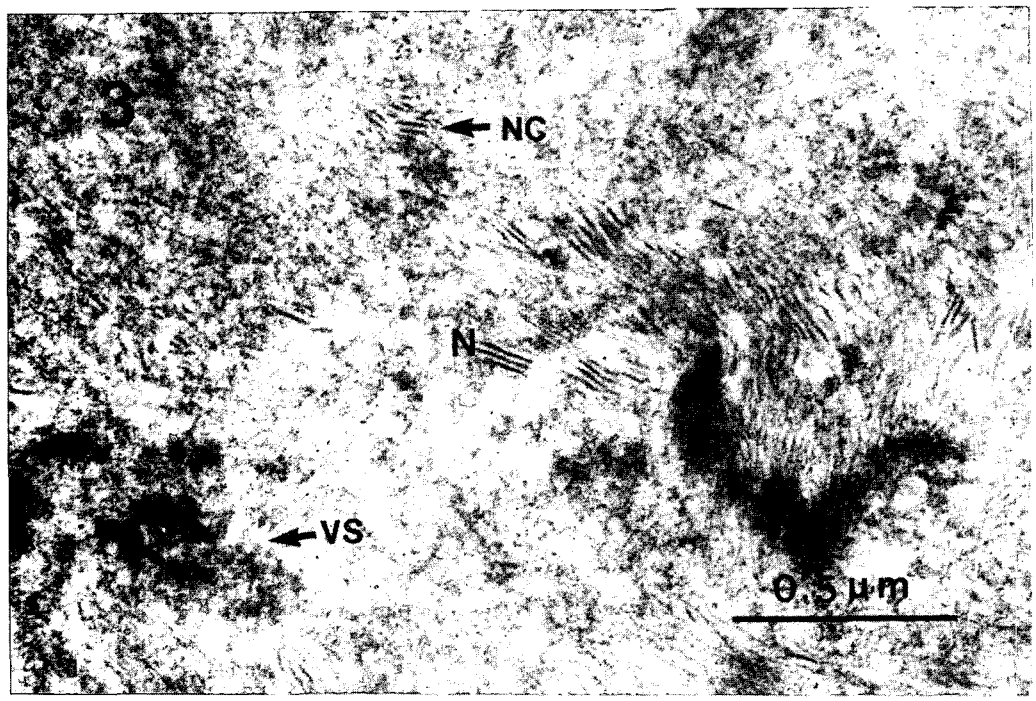
Fig. 2. The midgut cell of *D. spectabilis* larvae infected with *D. spectabilis* nuclear polyhedrosis virus. Virogenic stroma(VS) was formed in the nucleus. Other symbols are the same in Fig. 1.

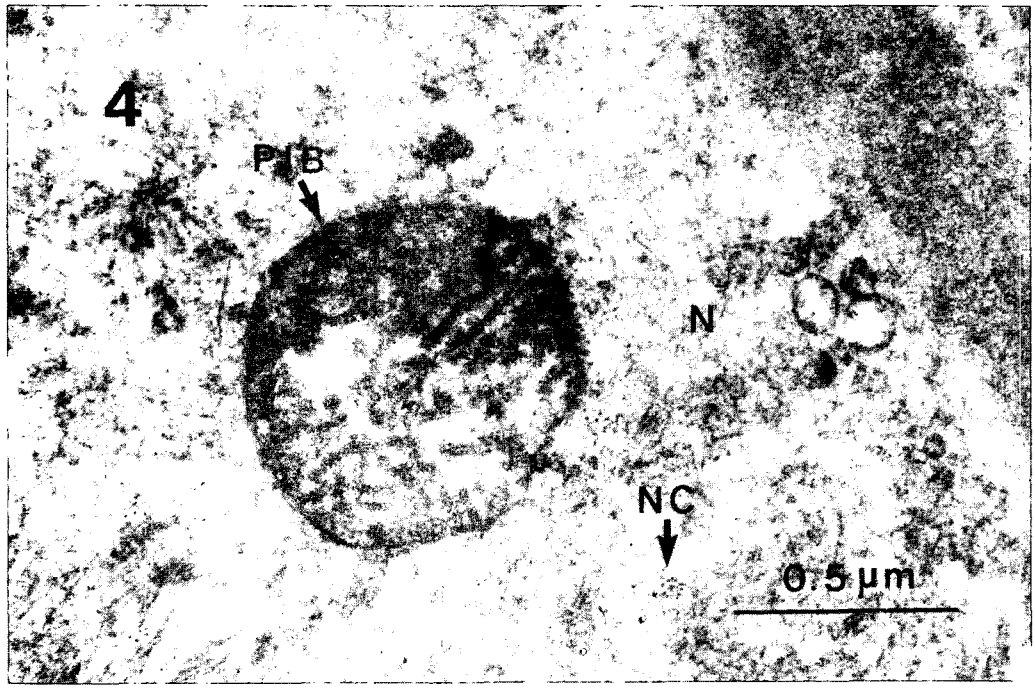
Fig. 3. Formation of nucleocapsids(NC) from the virogenic stroma(VS) in the midgut cells of *D. spectabilis* larvae. Other symbols are the same in Fig. 1 and 2.

Fig. 4. Formation of polyhedra(PIB) in the midgut cell of *D. spectabilis* larvae by infection of *D. spectabilis* nuclear polyhedrosis virus. Other symbols are the same in Fig. 2.


1
Mt
NM
N
1 μm


2
N
NM
VS
1 μm


3
NC
N
VS
0.5 μm


4
PM
N
NC
0.5 μm