Journal of Invertebrate Pathology **77,** 144–146 (2001)
doi:10.1006/jipa.2000.5006, available online at http://www.idealibrary.com on IDEAL®

NOTE

Replication of *Insect Iridescent Virus 6* in a Whitefly Cell Line

The whitefly, *Bemisia tabaci,* B biotype (syn. *B. argentifolii* Bellows and Perring) (Homoptera:Aleyrodidae), is a major problem in agroecosystems throughout tropical and subtropical regions of the world. Large populations of whiteflies reduce crop yields, cause contamination of plant parts with excreted honeydew, and act as vectors of Begomoviruses and other plant viruses that cause serious economic losses in vegetable and fiber crops (Brown *et al.,* 1995).

According to the Ecological Database of the World's Insect Pathogens (http://insectweb.inhs.uiuc.edu/Pathogens/EDWIP/), a pathogenic virus of whiteflies has not been reported to date. Although virus-like particles have been observed in *B. tabaci* male germ and cyst cells (Báo *et al.,* 1996) and in mycetomes and ovarian tissue (Costa *et al.,* 1996), whether the particles are of viral origin is not known. One candidate viral pathogen of whiteflies is the *Insect iridescent virus 6* (IIV-6), also known as the *Chilo iridescent virus.* This virus was originally isolated from the rice stem borer (*Chilo suppressalis*) (Lepidoptera) (Fukaya and Nasu, 1966), but it was previously demonstrated that the virus can replicate in leafhoppers, leafhopper cells, and planthopper tissue explants (Jensen *et al.,* 1972; Mitsuhashi, 1967). The ability to replicate in leafhopper cells raised the possibility that a whitefly cell line could also support IIV-6 replication. This report describes the productive infection of a whitefly cell line with IIV-6 using light and electron microscopy, fluorescent antibody staining, and Western blot analysis.

A recently established whitefly embryo cell line, designated BtB-2.97-Hunter & Polston (manuscript in preparation), was cultured in modified Liu and Black insect medium (Kimura, 1984). The BtB-2.97 cells were incubated at room temperature (20–22°C) and passed at a 1:3 ratio once per week. An IIV-6 isolate (obtained from J. Kalmakoff) was amplified in third-instar *Tricoplusia ni* larvae and purified using differential centrifugation (Marina *et al.,* 1999). The identity of the amplified virus isolate was confirmed by sequencing a portion of the major capsid protein gene (not shown). The virus solution was passed through a sterile 0.45-μm filter before infection of cells.

Initial attempts at infecting whitefly cells were made by adding a filtered IIV-6 suspension (0.2 μg protein $\cong$ 1×10^9 PFU) to 1.8×10^6 BtB-2.97 cells and by passing infected cell media (1:67 dilution) to additional whitefly cell cultures. All subsequent studies were made after an end point dilution titration method was developed for IIV-6 on BtB-2.97 cells (similar to Summers and Smith, 1987). Whitefly cells were fixed for transmission electron microscopy (3% glutaraldehyde, 2% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2, overnight at 4°C), postfixed (3 h in 1% OsO_4), and embedded in Spurr's resin using standard methods (Dawes, 1979).

Cells that were inoculated with IIV-6 developed cytopathic effects (CPE) that indicated the virus was replicating in the BtB-2.97 cell line. Infected cells became more rounded and developed inclusions that increased in size over the infection period (Figs. 1A, 1B). Examination of infected cells by electron microscopy revealed that an electron-dense virogenic stroma had formed and that the icosahedral virus accumulated only in the cytoplasm (Figs. 1C, 1D), which is characteristic of iridoviruses.

Immunofluorescent staining was used to detect virus structural proteins within the cells. Whitefly cells were grown on coverslips and infected with IIV-6 (multiplicity of infection (moi) of 10). The coverslips were removed at various times postinfection, fixed in 3.7% formaldehyde, and permeabilized for 10 min in acetone at −20°C. The cells were probed with an anti-IIV-6 antibody (a gift from Dr. J. Kalmakoff) for 30 min (1:500 dilution), then probed with an anti-rabbit antibody labeled with Alexa Fluor 488 (Molecular Probes Inc.) (30 min, 1:200 dilution), and counterstained with 300 nM 4′,6-diamidino-2-phenylindole (DAPI, Molecular Probes).

Structural proteins of IIV-6 were detected in BtB-2.97 cells by day 2 postinfection (p.i.) (Figs. 1E–1G). Initially the viral proteins were localized in small zones adjacent to the nucleus, but by day 3 p.i. the zone was larger and could be co-localized within new regions of nucleic acid using DAPI, a nucleic acid stain (not shown). This indicated that IIV-6 structural proteins were present in the virogenic stroma, the site of virus assembly. Although the heaviest staining localized to a defined area of virogenic stroma, much of the rest of the cytoplasm displayed a punctate staining pattern indicating that viral proteins were also scattered throughout the cytoplasm.

The expression of IIV-6 structural proteins in whitefly cells was confirmed by Western blot analysis (Fig.

0022-2011/01 $35.00

2). Whole-cell homogenate was made from infected cells (moi of 100) at various times postinfection. The cells were solubilized in buffer (50 mM Tris, pH 6.8, 0.1% SDS) and samples (10 μg each) were separated on an 11% polyacrylamide gel using SDS–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Immobilon P, Millipore Corp). IIV-6 proteins were probed using the anti-IIV-6 antibody, followed by an incubation with a secondary anti-rabbit antibody conjugated with alkaline phosphatase.

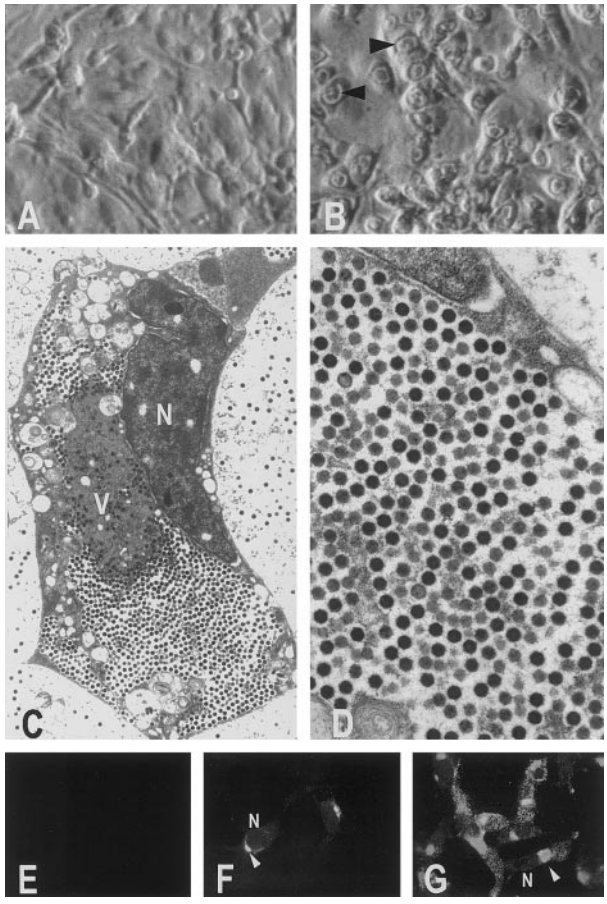

FIG. 1. Infection of whitefly cells with IIV-6. (A) Uninfected BtB-2.97 cells and (B) cells infected with IIV-6 at 11 days postinfection (relief phase optics, 400× magnification). Infected cells developed cellular inclusions (arrowhead). (C, D) Transmission electron micrographs of IIV-6-infected BtB-2.97 cells. (C) Virions in the cytoplasm and within the virogenic stroma (V). (N, nucleus; 8000× magnification). (D) A higher magnification of the same cell. Icosahedral virus particles densely packed in regions of the cytoplasm (26,250× magnification). (E–G) Immunofluorescent staining of IIV-6-infected BtB-2.97 cells. Whitefly cells were grown on coverslips, infected with IIV-6, and harvested at various time points. The coverslips were stained with anti-IIV-6 antibodies, detected with a secondary antibody (labeled with Alexa Fluor 488), and examined on a Zeiss Axioskop fitted with an epifluorescence illuminator. The locations of the virogenic stroma (arrowhead) and the nucleus (N) are indicated. (E) 1 day postinfection. (F) 2 days p.i. (G) 3 days p.i. Viral structural protein antigens were not detected in mock-infected cells (not shown) (375× magnification).

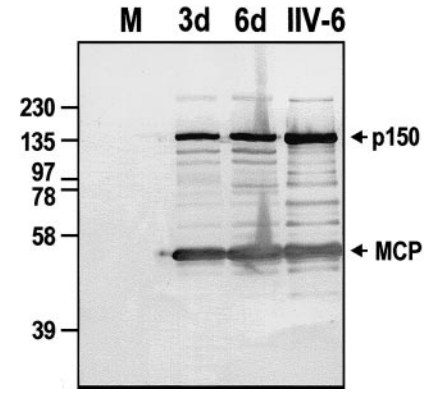

FIG. 2. Western blot analysis of infected cell homogenates. Ten micrograms of a mock-infected whitefly cell homogenate (M), IIV-6-infected whitefly homogenates at 3 and 6 days postinfection, and purified IIV-6 were separated by SDS–PAGE, blotted to PVDF, and probed with an IIV-6 antibody (rabbit polyclonal serum, 1:5000 dilution). The IIV-6 antibody was detected with an alkaline phosphatase-labeled secondary antibody (anti-rabbit) and the viral proteins were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium. Molecular weight markers (kDa) are indicated on the left side of the blot. MCP, IIV-6 major capsid protein; p150, 150-kDa MCP trimer.

The viral protein bands were visualized with the phosphatase substrate 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

Viral proteins, including the abundant major capsid protein, were expressed in whitefly cells by day 3 p.i., while the minor structural proteins became more abundant by day 6 p.i. (Fig. 2). Both forms of the MCP, the 55-kDa monomer and the 150-kDa trimer (Cerutti and Devauchelle, 1990; Davison *et al.*, 1992), were present in infected BtB-2.97 cells.

Insect iridescent virus 6 is the first virus shown to replicate in cells derived from a whitefly species. The establishment of an IIV-6 infection in whitefly cells is an important first step in utilizing the virus to study whitefly cells. Other insect viruses (e.g., baculoviruses) have been used as tools for examining various aspects of cell and molecular biology such as apoptosis, cytoskeletal movement and rearrangement, DNA replication, and gene expression (Miller, 1997). In addition, preliminary results indicate that IIV-6 can also infect whitefly nymphs (research in progress), which could provide a tool for studying whiteflies at the organismic level.

Key Words: insect iridescent virus 6; Chilo iridescent virus; iridovirus; Homoptera; Aleyrodidae; Bemisia; silverleaf whitefly.

We thank James Kalmakoff for providing the IIV-6 and antibody. We also thank N. Parks and S. Carrington-Hahn for their technical assistance and E. Davidson, M. Salvucci, and S. Lapointe for their helpful comments. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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Received July 26, 2000; accepted December 29, 2000; published online March 3, 2001

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