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TRANSFORMATION OF LEPIDOPTERAN AND COLEOPTERAN INSECT CELL LINES BY *GLYPTAPANTELES INDIENSIS* POLYDNAVIRUS DNA

D. GUNDERSEN-RINDAL,[1] D. E. LYNN, AND E. M. DOUGHERTY

U.S. Department of Agriculture, Agricultural Research Service, Insect Biocontrol Laboratory, Beltsville, Maryland 20705

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SUMMARY

Recently investigators showed that polydnavirus DNA from the parasitic wasp Glyptapanteles indiensis could transform gypsy moth *L. dispar* cell lines in vitro (McKelvey et al., 1996). Here we show GiPDV DNA is capable of transforming in vitro to varying degrees lepidopteran (IPLB-TN-R^2, IPLB-SF-21, IAL-PID2, IPLB-HvT1) and coleopteran (IPLB-DU182E) insect cell lines derived from various somatic tissue types. An insect cell line derived from dipteran *Aedes albopictus* (C7/10) could not be transformed with G. indiensis polydnavirus.

Key words: in vitro; parasitic wasp; Lymantria dispar.

INTRODUCTION

Cell lines have been established from a large number of insect species, predominantly from the orders Lepidoptera and Diptera (10). These cell lines have facilitated study of insect cellular physiology, endocrinology, and biochemistry. A variety of cell lines have been used for studies on insect viruses and have shown differing susceptibilities to infection (10). It has often been beneficial to manipulate insect viral replication in insect cell lines. For example, the well-known baculovirus expression vector system has been used extensively for foreign gene expression in insect cells in vitro (reviewed in 8). Recently, other insect viruses have been investigated for potential utility as vectors to introduce genetic material to insect cell genomes in vitro (5,14,18). However, stable transformation of insect tissues, in vivo or in vitro, has seldom been achieved (2).

Polydnaviruses, large multisegmented DNA viruses found only in the female reproductive tract of some hymenopteran parasitic wasp hosts, are injected during parasitization into a lepidopteran insect host where they do not replicate but disrupt the host immune system (9). Recently, polydnavirus DNA from the parasitic wasp Glyptupanteles indiensis (GiPDV) was shown to persist within the chromosomal DNA of a host gypsy moth L. dispar cell line, maintained essentially as a permanent part of replicating cells; thus, the cell line was "transformed" (14). It was believed that part of GiPDV DNA genome was maintained in transformed cells as integrated into cell chromosomal DNA (14). Hence, GiPDV DNA has potential use as a vector for *L.* dispar insect cell transformation. GiPDV DNA may also have utility for generalized insect cell transformation. In this study, we investigated the in vitro host range of GiPDV by assessing whether GiPDV DNA was capable of persisting not only in host *L.* dispar cell line DNA but also in several other nonhost insect cell lines derived from various somatic tissue types from lepidopteran and nonlepidopteran insects of agricultural and economic importance.

MATERIALS AND METHODS

Source of cell lines. Established insect cell lines IPLB-LdEp, IPLB-TN-R_2, IPLB-SF-21, IAL-PID2, IPLB-DU182E, IPLB-HvT1, IPLB-Tconl, IPLB-Tex2, IPLB-LdFB, and C7/10 were maintained by routine subculture in the laboratory. Their origins and properties are described in Table 1.

Generation of cell lines infected with polydnavirus. Insect cell lines listed in Table 1 were infected separately with polydnavirus (GiPDV), each from a single braconid *G. indiensis* adult female, as previously described (14). Briefly, two ovaries and the venom gland were dissected from a single wasp and placed into a well of a 24-well dish containing 40–60% confluent insect cells in 0.5 ml appropriate media listed in Table 1 (Excel1400 and IPL-52B, JRH Biosciences, Lenexa, KS; TNM-FH and Eagle's MEM, GIBCO/BRL, Gaithersburg, MD) with 25 µg gentamicin per ml. The calyx fluid and venom were released, mixed, and incubated with cells at 26" C. Infected cells were subcultured as determined by cell density.

Isolation and selection of a GiPDV clone comprising DNA maintained in GiPDV-infected L. dispar cellular DNA. A GiPDV plasmid clone library was generated by the method described by Albrecht et al. (1) in which GiPDV DNA fragments partially digested with *Eco*RI in the presence of ethidium bromide were inserted into the EcoRI site of the pSP64 vector (Promega Corp., Madison, WI) by electroporation. Ethidium bromide, which does not easily saturate superhelical DNA until it is nicked, was included in the GiPDV DNA digestion to increase the likelihood of cloning full ds circular DNA segments of the GiPDV genome. The library was screened for individual clones that represented GiPDV DNA capable of persisting in infected gypsy moth cells in vitro, which were selected by dot hybridization at 48° C with digoxigenin-labeled total GiPDV DNA, IPLB-LdEp cellular DNA, and IPLB-LdEp infected with GiPDV (IPLB-LdEp/Gi) DNAs separately as probes. Clones which hybridized strongly with GiPDV and IPLB-LdEp/Gi probes and did not hybridize at all with the IPLB-LdEp probe were selected for further analysis. Clone p157 (18.7 kb) (Fig. 1) hybridized strongly with GiPDV and IPLB-LdEp/Gi probes and showed no hybridization at all with the IPLB-LdEp probe. The annealed DNAs were visualized with CSPD (disodium **3-(4-**methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.$1_{3,7}$]decan]-4-yl) phenyl phosphate) (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Analysis of putatively transformed insect cell lines for GiPDV sequences by Southern blot. Total genomic nucleic acids were extracted from each noninfected and GiPDV-infected (putatively transformed) insect cell line by standard techniques (3). All cell lines had been passaged at least 10 times at the

[1]To whom correspondence should be addressed at U.S. Department of Agriculture, Insect Biocontrol Laboratory, Building 0llA, Room 214, BARC West, Beltsville, Maryland 20705; Email: dgunders@asrr.arsusda.gov.

TABLE 1

ORIGIN OF INSECT CELL LINES

Cell Line	Origin	Developed from	Medium	Reference
IPLB-LdEp	Lepidoptera	*Lymantria dispar,* gypsy moth embryos	ExCell400	(14)
IPLB-LdEp/Gi[a]			"	
IPLB-LdEIta	Lepidoptera	*Lymantria dispar,* gypsy moth embryos		(14)
IPLB-LdEIta/Gi				
IPLB-LdFB	Lepidoptera	*Lymantria dispar,* gypsy moth larval fat bodies	"	(10)
IPLB-LdFB/Gi				
IPLB-TN-R2	Lepidoptera	*Trichoplusia ni,* cabbage looper embryos	TNM-FH	(19)
IPLB-TN-R2/Gi			"	
IPLB-SF-21	Lepidoptera	*Spodoptera frugiperda,* fall armyworm pupa ovaries		(20)
IPLB-SF-2l/Gi			"	
IAL-PID2	Lepidoptera	*Plodia interpunctella,* Indian meal moth imaginal wing discs		(12)
IAL-PID2/Gi			"	
IPLB-HVT1	Lepidoptera	*Heliothis virescens,* tobacco budworm testes		(10)
IPLB-HvTl/Gi				
IPLB-DU182E	Coleoptera	*Diabrotica undecimpunctata,* corn rootworm embryos	IPL-52B	(13)
IPLB-DU182E/Gi				
IPLB-Tconl	Hymenoptera	*Trichogramma confusum,* trichogrammid wasp embryos	ExCell400	(11)
IPLB-Tcon1/Gi			"	
IPLB-Tex2	Hymenoptera	*Trichogramma exiguum,* trichogrammid wasp embryos		(11)
IPLB-Tex2/Gi				
C7/10	Diptera	*Aedes albopictus,* mosquito egg	Eagles MEM	(15)
C7/10/Gi				

[a]Cell lines labeled "/Gi" were infected with GiPDV and passaged weekly as described in the text.

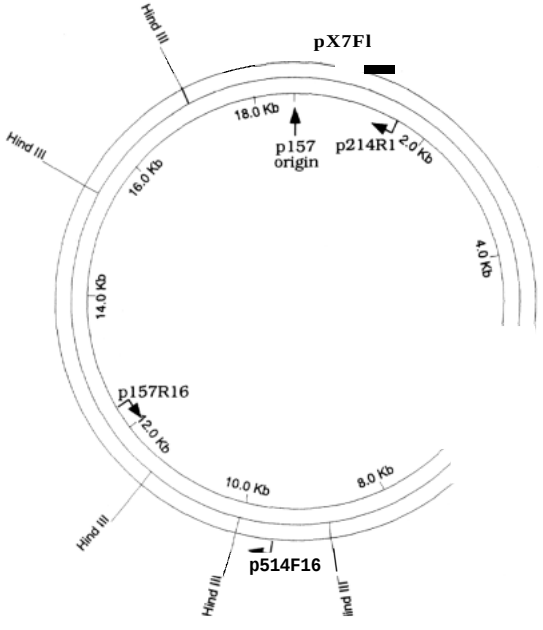

FIG. 1. Map of clone p157 (representing one circular segment of the GiPDV genome) showing *Hind*III restriction site locations and approximate locations of binding sites for primers p157F1, p214R1, p214F16, and p157R16. Approximate size in kilobase pairs is shown on the figure.

time nucleic acid was extracted. Genomic DNA (5 μg) from each sample was digested with restriction endonuclease *Hind*III (GIBCO/BRL), electrophoresed through a 0.8% agarose gel, and transferred to nylon membrane as described (16). Digoxigenin-labeled (Boehringer Mannheim Biochemicals) plasmid DNA from GiPDV clone p157 was used as a hybridization probe. Annealed DNAs were visualized with CSPD (Boehringer Mannheim Biochemicals). The temperature for hybridization was 40" C.

Analysis of putatively transformed insect cell line DNAs for GiPDV sequences by polymerase chain reaction (PCR). Several oligonucleotide primer pairs were designed for PCR on the basis of GiPDV sequences determined by direct sequencing of parts of GiPDV clone p157 and p214, a polymorphic clone of the same GiPDV dsDNA. Approximate locations within clone p157 of sequences complimentary to primer sequences are given on the map in Fig. 1. The oligonucleotide sequences of the designed primers were:

p157F1: 5'-CTA CGT CTA GCT TCT CAA G-3'

p214R1: 5'-CAT GAT TAA TAC TGT AAC ATC-3'

p214F16: 5'-CTA CAA GAT ACA GGT CAA TC-3'

p157R16: 5'-GCG GTG ATG ACG TAG GTG AC-3'

For PCRs, total genomic nucleic acids extracted from GiPDV and each noninfected and GiPDV-infected cell line were diluted in sterile deionized water to a final concentration of 20 ng/p1. Each 50-μ1 PCR reaction was performed with AmpliTaq Gold (Perkin-Elmer Cetus, Norwalk, CT), 0.2 μ*M* of each primer, and 200 μ*M* deoxynucleotide triphosphates. Thirty-five PCR cycles were conducted in an automated thermocycler (Hybaid, Teddington, United Kingdom). After a 10-min initial enzyme activation at 94" C, the following parameters were used: 1-min (2-min for the first cycle) denaturation at 94" C, 2-min annealing at 55" C, and 3-min primer extension (10-min in final cycle) at 72° C. Reactions devoid of template were included in each experiment as negative controls. PCR products were analyzed by electrophoresis through a 0.8% agarose gel followed by staining in ethidium bromide and visualization of DNA bands with a UV transilluminator.

RESULTS

Infection of lepidopteran and coleopteran cell lines derived from various tissues with GiPDV induced distinct, temporary changes in host cell morphology and growth rate. As compared with uninfected cells, cells infected with GiPDV showed a reduced rate of cell division and formed aggregates not normally seen in uninfected cells (data not shown). For all cell lines, the GiPDV-induced effects on

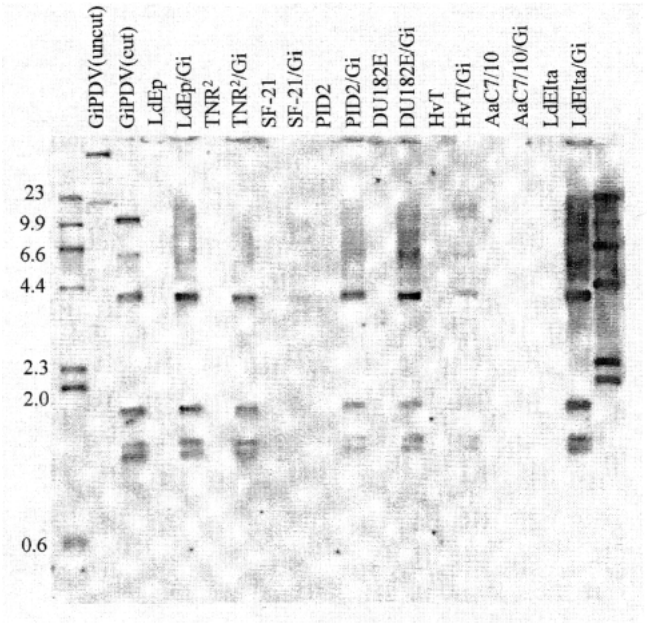

FIG. 2. Southern blot analysis of GiPDV-infected insect cell lines for GiPDV sequences. Genomic DNA from GiPDV virus, noninfected, and GiPDV-infected cell lines digested with HindIII was electrophoresed through a 0.8% agarose gel and transferred to nylon membrane as described (16). Digoxigenin-labeled DNA (BMB, Indianapolis, IN) from clone p157 was used as a hybridization probe. *Lanes* contain HindIII digested genomic DNA from GiPDV, uninfected insect cells, or GiPDV-infected insect cells as labeled. The faint 6-kb band in GiPDV HindIII digest disappears using higher stringency for hybridization. Abbreviations are as given in Table 1. Lanes *1* and 20 contain molecular size standard bands of 23, 9.9, 6.6, 4.4, 2.3, 2.0, and 0.6 kilobase pairs (from ***top*** to *bottom).*

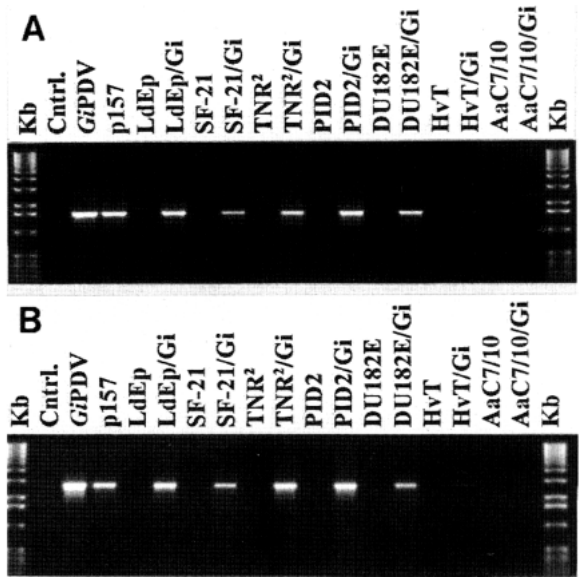

FIG. 3. Polymerase chain reaction (PCR) amplification of GiPDV sequences from infected and noninfected insect cell line DNAs with GiPDV-specific primer pairs *(A)* p157Fl/p214RI and *(B)* p214F16/p157R16. Abbreviations are as given in Table 1. Lanes **1** and *19* contain molecular size standard bands of 12.2, 11.2, 10.2, 9.2, 8.1, 7.1, 6.1, 5.1, 4.1, 3.1, 2.0, 1.6, 1.0, and 0.5 kilobase pairs (from *top* to *bottom).*

cell growth and morphology gradually dissipated through weekly passage of the cell lines. The growth rates of uninfected and infected cells were similar after 1–2 mo. (data not shown).

Infected insect cells were analyzed for the presence of GiPDV viral sequences with labeled GiPDV clone p157 DNA as a probe. This GiPDV clone represented part of the GiPDV DNA that is capable of persisting in the IPLB-LdEp cell line in vitro. In addition, clone p157 represented one complete circular segment of the GiPDV genome (D. Gundersen-Rindal and E. M. Dougherty, unpublished data). Within the GiPDV genome, this circular segment was unique as it hybridized only with relaxed circular and superhelical forms of a single GiPDV genome segment (Fig. 2, *lane* GiPDV uncut). No signal was detected from DNA of any uninfected cells when Southern-blotted, HindIII-digested DNAs were probed with labeled p157 DNA (Fig. 2). Signal was detected from GiPDV DNA and GiPDV-infected cell line DNAs from IPLB-LdEp/Gi, IPLB-SF-2l/Gi (faint), IPLB-TN-R^2/Gi, IAL-PID2/Gi, IPLB-DU182E/Gi, and IPLB-HvT1/Gi (faint), and IPLB-LdEIta/Gi (Fig. 2). No signal was detectable from GiPDV-infected mosquito cell line C7/10. Some GiPDV-infected cell lines, particularly IPLB-SF-21/Gi and IPLB-HvT1/Gi, hybridized much less strongly with p157 DNA than others. In all transformed cell lines, the GiPDV sequences appeared to be stably maintained, as several of the GiPDV-infected insect cell lines had been maintained for over 100 passages (IPLB-LdEp/Gi for over 300 passages), without any loss of detectable GiPDV signal, at the time genomic DNA was extracted.

PCR primer pairs p157Fl/p214R1 and p214F16/p157R16, designed for specific amplification of GiPDV sequences from two separate regions of the clone p157 circular segment (Fig. **1**), primed amplification of 1.7-kb and 2.8-kb DNA fragments, respectively, from GiPDV, GiPDV clone p157, and from IPLB-LdEp/Gi, IPLB-TN-R2/Gi, IPLB-SF-21/Gi, IAL-PID2/Gi, IPLR-DUl82E/Gi, and IPLB-HvTl (weak) GiPDV-infected cell line DNAs (Fig. 3*A* and *B*). These primers also primed amplification of 1.7-kb and 2.8-kb DNA fragments from IPLB-Tconl/Gi, IPLB-Tex2/Gi, and IPLB-FB/Gi GiPDV-infected cell line DNAs (data not shown). These GiPDV-specific primer pairs did not prime amplification from GiPDV-infected mosquito cell line C7/10 (Fig. **3** *A* and *B*, *lane* AaC7/10/Gi) or from any noninfected insect cell line DNA (Fig. 3*A* and ***B*).**

DISCUSSION

Systems for stable transformation of insect cells have been pursued because of their potential to advance fields such as agriculture and medicine. Routine gene transfer in insects has been possible only in members of the genus *Drosophila* (7), and methods have been actively sought for generalized transformation of other insect genomes. Insect cell culture provides ideal conditions for study and manipulation of insect genomes in vitro. Increased efforts have been made to isolate transposable and viral elements as vehicles for in vitro and in vivo genetic transformation of insect cells (2,5,7). Virus-based systems have been of particular interest for use in biotechnology for gene expression in vitro (5,7,8).

Polydnaviruses, found in the female reproductive tract of their braconid or ichneumonid parasitic wasp hosts, are injected along

with eggs into insect hosts during parasitization (4,6,9,17). A portion of the braconid G. indiensis polydnavirus genome is able to persist in the insect host *L.* dispar (gypsy moth) chromosome in vitro and is stably maintained, as described earlier by McKelvey et al. (14). We have found, by hybridization and PCR assays, that a portion of the GiPDV genome can also persist in vitro in infected insect cell lines derived from other Lepidoptera (*Spodoptera frugiperda, Trichoplusia ni, Plodia interpunctella,* and *Heliothis virescens*) and from the opteran *Diabrotica undecimpunctata.* These insects do not normally encounter GiPDV in nature, but their cells are apparently susceptible to various degrees to GiPDV infection in vitro. Similar restriction patterns were obtained among the diverse infected insect cell lines on Southern blots with GiPDV-specific clone p157 used as a probe, though none of the infected cell line DNAs contained the largest GiPDV HindIII fragment (approximately 11 kb) (Fig. 2). Similar PCR-amplified GiPDV sequences among infected insect cell lines (Fig. 3) indicated that a similar portion(s) of the GiPDV genome was maintained in these cell lines. It is possible these GiPDV sequences are maintained in vitro in episomal fashion in the transformed insect cell lines described. However, we believe the GiPDV DNA is potentially integrated in the chromosomal DNA of transformed insect cell lines. Evidence shown previously (14) in which clones containing both IPLB-LdEp cellular and GiPDV viral DNA sequences were isolated from GiPDV-infected IPLB-LdEp cells suggested that GiPDV DNA is capable of integration into the *L. dispar* cell line IPLB-LdEp chromosome. If GiPDV DNA were also capable of integration in other insect cells, a similar GiPDV restriction pattern observed among transformed insect cell lines could suggest a potentially similar site of GiPDV integration within the chromosome of each insect cell line. Absence of the largest HindIII fragment of the GiPDV circular segment represented by clone p157 in transformed insect cell DNAs indicated that a potential site of integration may be present within this GiPDV HindIII fragment (Fig. 2). Studies are currently underway in our laboratory to isolate potential site(s) of integration in several of these transformed insect cell lines. GiPDV DNA apparently cannot persist in vitro in infected cells derived from the dipteran *Aedes albopictus* (C7/10) based on hybridization and PCR assays.

Because of its potential use in biotechnological applications, we are very interested in determining the potential in vitro host range of GiPDV. Our studies have shown a wide range of lepidopteran cell lines permissive to infection with GiPDV DNA. These included cells from three families which had been isolated from five different tissue sources. More notable is our evidence that the GiPDV DNA could persist in coleopteran cells (Diabrotica undecimpunctata IPLB-DU182E). This suggests a very broad potential in vitro host range. However, the lack of persistence in dipteran (*Aedes albopictus* C7/10 mosquito cells) suggests there are limits to the GiPDV DNA host range. Examination of other dipteran cell lines should determine if at least some species within this order of insects are susceptible.

The polydnavirus isolated from G. indiensis has potential to be developed for generalized insect cell transformation. The transformed insect cell lines described here were derived from somatic tissues from insect pests of economic and agricultural importance. If GiPDV DNA were developed as a viral vector, such a vector should be functional in these lepidopteran and coleopteran insect cell lines. If a GiPDV-based vector could be introduced into germline cells, there would also be potential for generating transgenic insects for use in biologically-based control efforts.

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