JOURNAL OF VIROLOGY, Nov. 2001, p. 11157–11165 Vol. 75, No. 22
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.22.11157–11165.2001

Genome Sequence of a Baculovirus Pathogenic for *Culex nigripalpus*

C. L. AFONSO,[1]* E. R. TULMAN,[1] Z. LU,[1] C. A. BALINSKY,[1] B. A. MOSER,[2] J. J. BECNEL,[2] D. L. ROCK,[1] AND G. F. KUTISH[1]

Plum Island Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York 11944,[1] and Center for Medical, Agricultural and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, Florida 32604[2]

Received 29 May 2001/Accepted 9 August 2001

In this report we describe the complete genome sequence of a nucleopolyhedrovirus that infects larval stages of the mosquito *Culex nigripalpus* (CuniNPV). The CuniNPV genome is a circular double-stranded DNA molecule of 108,252 bp and is predicted to contain 109 genes. Although 36 of these genes show homology to genes from other baculoviruses, their orientation and order exhibit little conservation relative to the genomes of lepidopteran baculoviruses. CuniNPV genes homologous to those from other baculoviruses include genes involved in early and late gene expression (*lef-4*, *lef-5*, *lef-8*, *lef-9*, *vlf-1*, and *p47*), DNA replication (*lef-1*, *lef-2*, *helicase-1*, and *dna-pol*), and structural functions (*vp39*, *vp91*, *odv-ec27*, *odv-e56*, *p6.9*, *gp41*, *p74*, and *vp1054*). Auxiliary genes include homologues of genes encoding the p35 antiapoptosis protein and a novel insulin binding-related protein. In contrast to these conserved genes, CuniNPV lacks apparent homologues of baculovirus genes essential (*ie-1* and *lef-3*) or stimulatory (*ie-2*, *lef-7*, *pe38*) for DNA replication. Also, baculovirus genes essential or stimulatory for early-late (*ie-1*, *ie-2*), early (*ie-0* and *pe-38*), and late (*lef-6*, *lef-11*, and *pp31*) gene transcription are not identifiable. In addition, CuniNPV lacks homologues of genes involved in the formation of virogenic stroma (*pp31*), nucleocapsid (*orf1629*, *p87*, and *p24*), envelope of occluded virions (*odv-e25*, *odv-e66*, *odv-e18*), and polyhedra (*polyhedrin*/*granulin*, *p10*, *pp34*, and *fp25k*). A homologue of gp64, a budded virus envelope fusion protein, was also absent, although a gene related to the other category of baculovirus budded virus envelope proteins, Ld130, was present. The absence of homologues of occlusion-derived virion (ODV) envelope proteins and occlusion body (OB) protein (polyhedrin) suggests that both CuniNPV ODV and OB may be structurally and compositionally different from those found in terrestrial lepidopteran hosts. The striking difference in genome organization, the low level of conservation of homologous genes, and the lack of many genes conserved in other baculoviruses suggest a large evolutionary distance between CuniNPV and lepidopteran baculoviruses.

The family *Baculoviridae* is a large and diverse family of occluded viruses with double-stranded DNA genomes that are pathogenic for insects, particularly of the lepidopteran, hymenopteran, and dipteran orders (23). They are divided into two genera: the nucleopolyhedroviruses (NPVs), which have large occlusion bodies (OBs) containing numerous virions (55), and the granuloviruses (GVs), which normally have single virions occluded within small granular OBs (70). Recently, the genome sequences of six NPVs and two GVs pathogenic for lepidoptera have been described (14, 34). In contrast, only limited information on the genes from dipteran and hymenopteran baculoviruses has been reported (42, 75).

Because a number of dipteran (mosquito) species are important vectors for a variety of human and veterinary diseases, extensive investigations to identify pathogens of these insects have been undertaken. The first mosquito baculovirus (AesoNPV) was isolated from *Aedes sollicitans* in Louisiana in 1969 (15), and over the following 20 years NPVs were isolated from 10 additional mosquito species of the genera *Aedes*, *Anopheles*, *Culex*, *Psorophora*, *Uranotenia*, and *Wyeomyia* (23). These genera are all members of the family *Culicidae*, which contains approximately 3,500 species. Because of the rarity of NPV isolation from mosquitoes and the difficulty of transmission in the laboratory, few cytopathological and morphological studies are available (24, 25, 66). However, the isolation and characterization of the conditions for virus transmission were recently reported for an NPV pathogenic for *Culex nigripalpus* (6, 42).

CuniNPV is highly pathogenic for *C. nigripalpus* and *Culex quinquefasciatus*, both of which are important vectors of St. Louis and Eastern encephalitis viruses (42), and it is responsible for epizootics in field populations of *C. nigripalpus* larvae. CuniNPV development is restricted to the nuclei of midgut epithelial cells in the gastric caeca and posterior stomach. As in other mosquito baculoviruses, CuniNPV has a double-stranded DNA genome that replicates in the nuclei and is packaged into rod-shaped singly enveloped nucleocapsids (42). It has two virion phenotypes, an occluded form (occlusion-derived virion [ODV]) that initiates infection in the midgut epithelial cells and a budded form (BV) that spreads the infection within the midgut. Like NPVs, the OBs are found exclusively in the nuclei of infected cells, but unlike NPVs, they are globular, not polyhedral. They are similar in size to GV OBs, but they typically contain four to eight virions, whereas each GV OB contains one or two virions. Additionally, Cuni-NPV OBs lack the envelope which surrounds the polyhedra of lepidopteran baculoviruses (42). Virion morphology and

* Corresponding author. Mailing address: Plum Island Animal Disease Center, P.O. Box 848, Greenport, NY 11944-0848. Phone: (631) 323-3011. Fax: (631) 323-3044. E-mail: cafonso@piadc.ars.usda.gov.

phylogenetic analysis of p74 and DNA polymerase led to the suggestion that this virus may represent a new genus, distinct from NPVs and GVs (42). In this report, we describe the complete genome sequence of CuniNPV and compare it to the genomes of other baculoviruses.

MATERIALS AND METHODS

CuniNPV DNA isolation, cloning, and sequencing. CuniNPV was obtained from field-collected *C. nigripalpus* larvae isolated in 1997 in Gainesville, Fla. (6, 42), and amplified in laboratory colonies of *C. nigripalpus* and/or *C. quinquefasciatus* larvae following the procedures of Moser et al. (42). CuniNPV genomic DNA was obtained from viruses at the eighth mosquito passage. DNA was purified from OBs which were extracted from infected larvae by homogenization followed by differential centrifugation (42). Random DNA fragments of 1.5 to 6.5 kbp were obtained by incomplete enzymatic digestion with *Tsp*509I endonuclease (New England Biolabs, Beverly, Mass.). DNA fragments were cloned into the dephosphorylated *Eco*RI site of pUC19 plasmids and grown in *Escherichia coli* DH10B cells (GibcoBRL, Gaithersburg, Md.). pUC19 plasmids were purified using alkaline lysis according to the manufacturer's instruction (Eppendorf 5 Prime, Boulder, Colo.). DNA templates were sequenced from both ends with M13 forward and reverse primers using dideoxy chain terminator sequencing chemistries (60) and the Applied Biosystems PRISM 3700 automated DNA sequencer (PE Biosystems, Foster City, Calif.). Chromatogram traces were basecalled with Phred (21, 22) and assembled with Phrap (21). Confirmatory assemblies were performed with The Institute for Genomic Research (67) and CAP3 (36) sequence assembly programs using quality files and clone length constraints (67). Quality files and default settings were used to produce a consensus sequence which was manually edited using the Consed sequence editor (29). An inconsequential degree of sequence variation was observed among sequenced clones within poly(A) stretches or short repeated motifs. This minor variation occurred in noncoding regions and did not affect sequence assembly. The final circular DNA consensus sequence represented on average eightfold redundancy at each base position and had an estimated error rate of 0.4/10 kbp.

DNA sequence analysis. Genome DNA composition, structure, repeats, and restriction enzyme patterns were analyzed as previously described (1, 2, 42). Open reading frames (ORFs) longer than 30 codons with a methionine start codon (64, 65) were evaluated for coding potential using the Hexamer (ftp.sanger.ac.uk/pub/rd) and Glimmer (59) computer programs. Minor ORFs (those completely contained within larger ORFs) were excluded. *bro* gene family analysis was done with the MEME (19) and CAP software programs (68). Early and late promoter sequences were identified from regions located upstream (100 bp) of initiation codons as described by Kuzio et al. (39). DNA repeats were identified using the EMBOSS computer programs (Palindrome, Inverted, Tandem, Quicktandem, and Dotplot) (ftp: uk.embnet.org/pub/EMBOSS) and Blast (3). Protein homology searches were conducted using Blast (3), PsiBlast (4), FASTA (49), and HMMER (20, 63) programs with the databases PROSITE, Pfam, Prodom, Sbase, Blocks, Domo, and GenBank (11). Blast analysis was done on nonredundant databases (7–27-01). GCG (19), MEMSAT (10), Psort (43), and SAPS (38) programs were used for general analysis, membrane prediction, and physical characterization of proteins.

Nucleotide sequence accession number. The CuniNPV genome sequence has been deposited in GenBank under accession no. AF403738.

RESULTS AND DISCUSSION

Genome features. The CuniNPV genome is a circular double-stranded DNA molecule of 108,252 bp with a G+C content of 50.9%. This size is in good agreement with a previous restriction enzyme size estimate of 105 to 110 kbp (42). The CuniNPV genome contains densely arranged nonoverlapping clusters of 2 to 10 ORFs that are oriented in both directions. For descriptive purposes, we have presented the CuniNPV in a linearized form. The locations, sizes, and positions of ORFs are shown in Fig. 1 and Table 1. The CuniNPV genome contains 252 ORFs of 60 or more codons, of which 109 are likely to encode proteins (Table 1). Forty-six putative CuniNPV genes contain typical early and late baculovirus promoters upstream from the initiation codon (Table 1) (39).

The CuniNPV genome contains four putative homologous regions (*hr*s) composed of 64- to 85-bp repeats located in intergenic regions which contain inverted repeats but lack sequence similarity to *hr*s from other baculoviruses (Fig. 2). Five copies of an imperfect 85-bp repeat motif (*hr1*) are located at positions 49149 to 49571. Imperfect and incomplete related sequences are also present at nucleotide positions 37792 to 37938 (*hr2*), 68533 to 68791 (*hr3*), and 75525 to 75600 (*hr4*). Homologous regions have been identified in all baculovirus genomes sequenced. These are composed of sets of closely related repeated sequences present at several locations throughout the genome. Evidence suggests that *hr*s may function as viral origins of replication (48) and enhancers of early gene expression (30). All previously sequenced baculovirus genomes contain 4 (*Plutella xylostella* GV) to 13 (*Lymantria dispar M*NPV [Ld*M*NPV]) *hr*s located in multiple genomic locations (32, 39). They are composed of direct DNA repeats and imperfect DNA palindromic sequences. In general, *hr*s share significant intragenomic sequence homology, although they may show very low homology between viruses (32, 33, 48, 53).

Gene categories. Gene order and orientation are not conserved between the CuniNPV genome and genomes of baculoviruses infecting lepidopteran species (34). Only 36 of the 109 putative CuniNPV genes demonstrate clear homology to genes from other baculoviruses, implying that the core of conserved baculovirus genes may be smaller than the previously suggested 65 genes (14) or that there has been such extensive evolution that gene relatedness cannot be determined. Seventy-two CuniNPV ORFs show no homology to any other known baculovirus ORFs. In addition, the amino acid conservation between CuniNPV and lepidopteran baculovirus ORFs is low (18 to 54%), with an average of 28% identity to *Autographa californica* NPV (Ac*M*NPV) (34). This is in contrast with homologues of *Xestia c-nigrum* and *P. xylostella* GVs, which show about 33% amino acid identity with the corresponding ORFs in Ac*M*NPV (32, 33).

Early transcription. CuniNPV lacks homologues of the lepidopteran baculovirus early transcription factors and regulators *ie-0*, *ie-1*, *ie-2*, and *pe38* (34). Promoters of early baculovirus genes are transcribed by a combination of host RNA polymerase II and virally encoded transcription factors (26). The absence of homologues of these genes may indicate that CuniNPV is dependent on host factors for early transcription or that unique CuniNPV genes are involved in these functions. Alternatively, conservation of certain CuniNPV genes may be extremely low, thus making identification of homologues of other baculoviruses impossible.

DNA replication. CUN045 (*lef-1*), CUNO25 (*lef-2*), CUN089 (*helicase-1*), CUN091 (*dna-pol*), CUN054 (*alk-exo*), and CUN018 (*vlf-1*) are homologues of genes likely involved in DNA replication (40). Significant amino acid changes in these homologues and the absence of a number of essential and nonessential DNA replication genes distinguish CuniNPV from lepidopteran baculoviruses.

In addition to DNA polymerase (CUN091), which was described previously (42), other CuniNPV genes implicated in DNA replication show significant differences from their baculovirus homologues. CUN045 (*lef-1*) has a 58-amino-acid deletion at the amino terminus and a 31-amino-acid insertion in

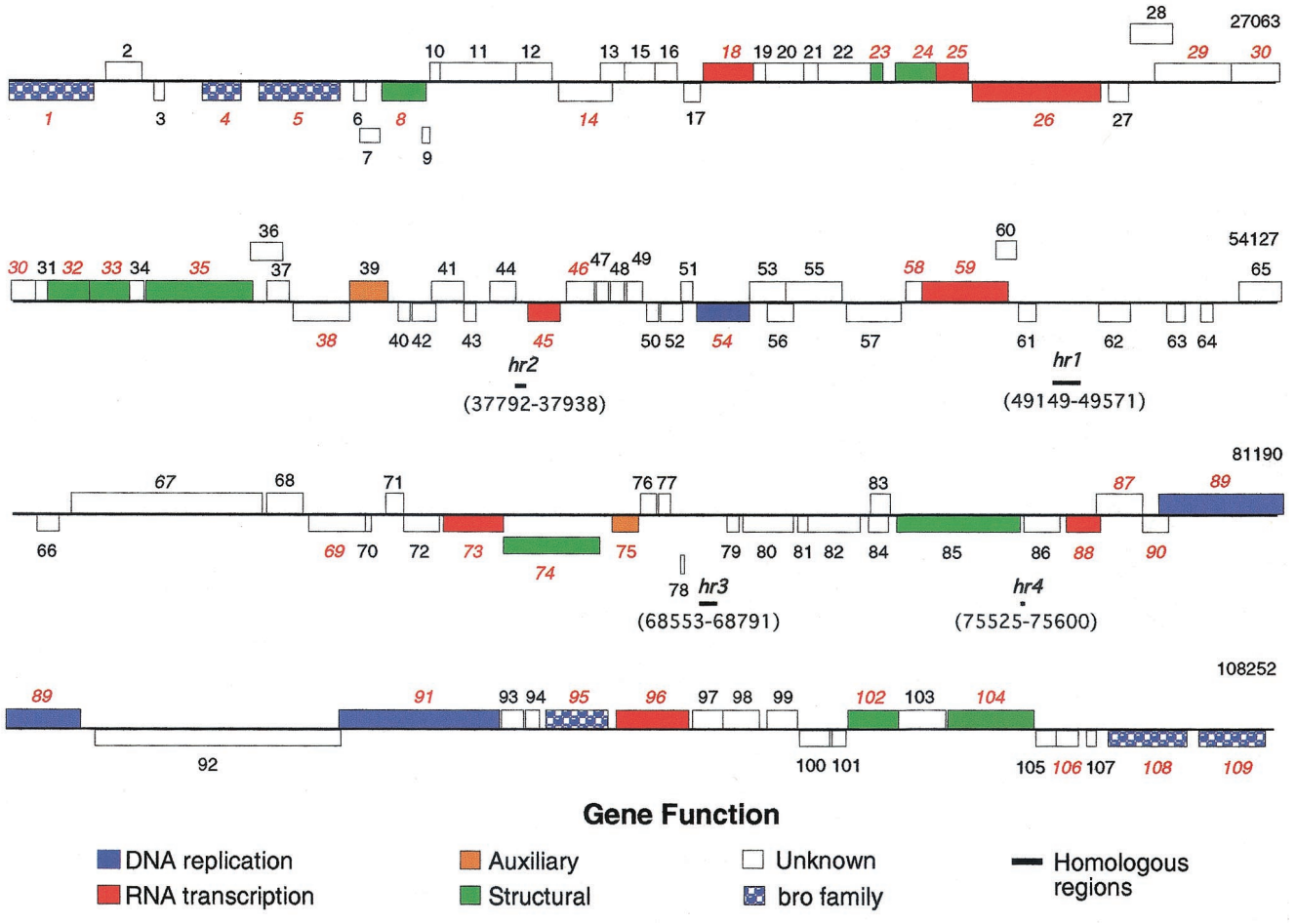

FIG. 1. Linear map of the CuniNPV genome. ORFs are numbered from left to right based on methionine initiation codon position. ORFs transcribed to the right are located above horizontal lines; ORFs transcribed to the left are below. Baculovirus homologues are indicated with red italicized numbers. Genes with related functions and members of gene families are colored according to the key.

the central part of the ORF in comparison to its closest homologue (Table 1) (5). CUN025 (*lef-2*) contains an N-4 cytosine-specific methylase Prosite signature (PS00093) at its amino-terminal end which is absent in other baculovirus LEF-2 proteins. In prokaryotes, N-4 cytosine-specific DNA methylases (EC 2.1.1.113) methylate the amino group at the C-4 position of cytosines in DNA, thus protecting DNA from cleavage by type II restriction enzymes. An N-4 cytosine-specific methylase signature motif may be the remnant of an ancestral restriction modification mechanism or a may indicate a dual function for the *lef-2* homologue. Active restriction modification systems have been found in large DNA viruses of the chlorella algae but not in baculoviruses (51). CUN054 (*alk-exo*) lacks 68 amino acids at the carboxy terminus in comparison to its closest homologue (Table 1) (40).

CUN089 (*helicase-1*) has statistically significant amino acid identity ($P = 10^{-13}$) to the *helicase-1* gene of *Choristoneura fumiferana* NPV (Table 1) and contains regions of similarity to baculovirus helicase domains I to IV (35). Interestingly, one of the most conserved regions corresponds to a *helicase-1* domain involved in host range specificity (17). In comparison to the *helicase-1* of other baculoviruses, CUN089 has low amino acid identity (Table 1) and lacks domain VI, the nucleotide binding motif, and the leucine zipper DNA binding motif. It also has 97 additional amino acids in the amino-terminal region.

CuniNPV lacks recognizable homologues of *lef-3*, which has the properties of a single-stranded-DNA binding protein and is involved in the transport of helicase into the nucleus (71). It should be noted, however, that *lef-3* and the early and late gene activator *ie-1* are very poorly conserved in baculoviruses (32, 33, 34). Other baculovirus genes apparently lacking are present only in some baculoviruses and include *dbp* (DNA binding protein), *lef-7*, *pcna* (proliferating cell nuclear antigen), *DNA ligase*, *rnr1* and *rnr2* (large and small subunits of ribonucleotide reductase), and *dUTPase* (34, 50).

Late and very late transcription. CUN096 (*lef-4*), CUN026 (*lef-8*), CUN059 (*lef-9*), and CUN073 (*p47*) are homologues of genes (Table 1) that encode a multisubunit RNA polymerase involved in late transcription (31, 41). In addition, CUN088 (*lef-5*) has been implicated in late gene transcription (52). CUN018 is similar to very late transcription factor 1 (*vlf-1*), the major transactivator of very late gene expression (41, 72–74).

In addition, a number of genes have been implicated in transcription by means of a transfection-based transient gene

TABLE 1. CuniNPV ORFs

ORF	Nucleotide position	Length (aa)[a]	Best match				ORF		Promoter type[c]	Comments[d]
			Accession no.[b]	Species	Blast score	Length (aa % identity)	Ac*M*NPV	XcGV		
CUN001	1849–71	593	AF162221	*Xestia c-nigrum* GV	327	451 (26)	2	60		*bro*, ATP_GTP-A motif
CUN002	2159–2827	223							L	ATP_GTP_A motif
CUN003	3366–3142	75								
CUN004	5016–4147	290								*bro*
CUN005	7085–5346	580	AF271059	*Helicoverpa armigera* NPV	237	355 (24)	2	60		*bro*
CUN006	7664–7362	101								
CUN007	7986–7354	211								
CUN008	8965–7979	329	AF169823	*Spodoptera exigua* NPV	144	246 (28)	54	175	E	*vp1054*, virion associated, assembly
CUN009	9053–8814	80							L	
CUN010	9054–9257	68								
CUN011	9261–10898	546							L	
CUN012	10919–11629	237							L	
CUN013	12743–13183	147								
CUN014	12888–11779	370	L22858	*Autographa californica* NPV	134	137 (26)	92	101		
CUN015	13193–13888	232							L	
CUN016	13891–14277	129							E	
CUN017	14829–14347	161							L	
CUN018	14847–15920	358	X71415	*Autographa california* NPV	250	327 (26)	77	123	L	*vlf-I*, very late expression factor I
CUN019	15940–16203	88							L	
CUN020	16203–16997	265							L	
CUN021	17019–17321	101								
CUN022	17321–18466	382								
CUN023	18476–18670	65	AF271059	*Helicoverpa armigera* NPV	107	48 (54)	100	94	L	*p6.9*, SR repeat, DNA binding
CUN024	19004–19870	289	P35840	*Lymantria dispar* NPV	139	291 (25)	89	111	L	*p39-capsid*, late coat protein
CUN025	19839–20513	225	U75930	*Neodiprion sertifer* NPV	93	173 (27)	6	35		*lef-2*, late expression factor 2, N4_Mtase motif
CUN026	23335–20570	922	AF162221	*Xestia c-nigrum* GV	553	865 (26)	50	148	E	*lef-8*, late transcription, Rna_Pol_Beta motif
CUN027	23918–23493	142								
CUN028	24026–24877	284								
CUN029	24534–26102	523	AF081810	*Lymantria dispar* NPV	573	488 (32)	119	84	L	
CUN030	26108–27529	474	AF169823	*Spodoptera exigua* NPV	98	182 (28)	142	13	L	
CUN031	27568–27795	76							L	
CUN032	27856–28659	268	AF081810	*Lymantria dispar* NPV	85	250 (22)	144	112		*odv-ec27*, occlusion-derived envelope
CUN033	28666–29523	286	AF169823	*Spodoptera exigua* NPV	64	186 (20)	80	121	L	*gp41*, tegument protein
CUN034	29526–29849	108							L	
CUN035	29909–32131	741	AF081810	*Lymantria dispar* NPV	639	510 (31)	83	118		*vp91-capsid*, virion protein
CUN036	32145–32780	212							L	
CUN037	32501–32929	143								
CUN038	34228–33020	403	AF081810	*Lymantria dispar* NPV	992	371 (52)	22	45		
CUN039	34250–34963	238	AF236641	*Spodoptera frugiperda* (armyworm)	145	215 (24)			L	
CUN040	35503–35207	99								
CUN041	36007–36579	191								
CUN042	36062–35547	172								
CUN043	36937–36656	94								
CUN044	37270–37791	174								
CUN045	38765–38061	235	AF271059	*Helicoverpa armigera* NPV	180	149 (32)	14	82		*lef-1*, late expression factor 1
CUN046	38897–39505	203	AF169823	*Spodoptera exigua* NPV	319	203 (31)	115	32	L	
CUN047	39510–39752	81								
CUN048	39764–40114	117							L	
CUN049	40171–40491	107							L	
CUN050	40861–40538	108								
CUN051	41329–41586	86								
CUN052	41384–40854	177								

Continued on facing page

TABLE 1—*Continued*

ORF	Nucleotide position	Length (aa)[a]	Best match				ORF		Promoter type[c]	Comments[d]
			Accession no.[b]	Species	Blast score	Length (aa % identity)	Ac*M*NPV	XcGV		
CUN053	42768–43583	272								
CUN054	42769–41669	367	AF271059	*Helicoverpa armigera* NPV	245	295 (28)	133	145		*alk-exo*, DNA processing, exonuclease
CUN055	43559–44716	386							L	
CUN056	43726–43193	178								
CUN057	46055–44826	410							E	
CUN058	46089–46499	137	AF037358	*Epiphyas postvittana* NPV	98	109 (26)	68	135		
CUN059	46471–48240	590	L33180	*Bombyx mori* NPV	303	388 (23)	62	139		*lef-9*, transcription, RNA polymerase motif
CUN060	48054–48443	130								
CUN061	48917–48477	147								
CUN062	50968–50222	249								
CUN063	52057–51665	131								
CUN064	52686–52345	114							L	
CUN065	53089–54120	344							E	
CUN066	55021–54539	161								
CUN067	55312–59382	1357							L	
CUN068	59508–60233	242							E	
CUN069	61562–60336	409	AF162221	*Xestia c-nigrum* GV	93	273 (25)	109	53		
CUN070	61746–61549	66							L	
CUN071	62028–62390	121							E	
CUN072	63185–62421	255							E	
CUN073	64553–63237	439	AF081810	*Lymantria dispar* NPV	171	241 (25)	40	78		*p47*, transcription regulator
CUN074	66537–64495	681	AF271059	*Helicoverpa armigera* NPV	1018	634 (35)	138	77	L	*p74*, envelope protein
CUN075	67409–66858	184	L22858	*Autographa californica* NPV	55	172 (18)	135			*p35*, apoptosis inhibitor
CUN076	67480–67809	110								
CUN077	67883–68092	70								
CUN078	68478–68173	102								
CUN079	69621–69310	104								
CUN080	70706–69624	361								
CUN081	71035–70820	72								
CUN082	72206–71031	392								
CUN083	72412–72774	121								
CUN084	72722–72348	125							E	
CUN085	75563–72918	882								Occlusion body
CUN086	76454–75669	262							E	ATP_GTP_A motif
CUN087	77217–78125	303	U75930	*Orgyia pseudotsugata* NPV	268	288 (34)	98	96	L	
CUN088	77267–76470	266	AF121349	*Neodiprion sertifer* NPV	96	200 (23)	99	95		*lef-5*, late expression factor
CUN089	78649–82644	1332	AF127530	*Choristoneura fumiferana* NPV	219	855 (20)	95	98	L	*helicase-1*, DNA replication
CUN090	78756–78151	202	L22858	*Autographa californa* NPV	216	169 (29)	96	97	L	
CUN091	88220–91633	1138	AF215639	*Spodoptera littoralis* NPV	473	636 (27)	65	132		*dna-pol*, DNA replication, polymerase
CUN092	88221–82915	1769							L	
CUN093	91721–92149	143								
CUN094	92213–92479	89								
CUN095	92641–93981	447	AF162221	*Xestia c-nigrum* GV	89	89 (31)	2	60		*bro*
CUN096	94194–95684	497	AF162221	*Xestia c-nigrum* GV	230	493 (25)	90	110		*lef-4*, transcription
CUN097	95822–96598	259							L	
CUN098	96469–97212	248							L	
CUN099	97438–98010	191								
CUN100	98824–98075	250								
CUN101	99108–98743	122								
CUN102	99162–100244	361	Q83953	*Orgyia pseudotsugata* NPV	318	302 (33)	148	15	L	*odv-e56*, envelope
CUN103	100301–101215	305							E/L	
CUN104	101319–103079	587	AE002638	*Drosophila melanogaster*	184	250 (24)	23	27	L	*Ld130*, envelope fusion protein
CUN105	103598–103164	145							L	
CUN106	104155–103598	186	AF162221	*Xestia c-nigrum* GV	111	139 (23)	81	120		
CUN107	104512–104261	84								

Continued on following page

TABLE 1—*Continued*

ORF	Nucleotide position	Length (aa)[a]	Best match				ORF		Promoter type[c]	Comments[d]
			Accession no.[b]	Species	Blast score	Length (aa % identity)	Ac*M*NPV	XcGV		
CUN108	106474–104672	601	AF162221	*Xestia c-nigrum* GV	354	449 (27)	2	60		*bro*, ATP_GTP_A motif
CUN109	108129–106660	490	AF162221	*Xestia c-nigrum* GV	138	198 (23)	2	60		*bro*, ATP_GTP_A motif

[a] aa, amino acids.
[b] Accession numbers are from the GenBank or SwissProt databases.
[c] Putative promoter. E, early; L, late.
[d] Function was deduced from the degree of amino acid similarity to known genes or by the presence of PROSITE signatures.

expression assay (52). Although CuniNPV contains the minimal complex necessary for late polymerase activity (*lef-4*, *lef-8*, *lef-9*, and *p47*) (31, 41), it lacks homologues of other lepidopteran baculovirus expression factors which are required for optimum levels of late transcription (*lef-6*, *lef-10*, *lef-11*, *lef-12*, and *pp31* [*39k*]) (Table 2). Some of the transcription factors absent in CuniNPV are species specific (*lef-7*, *lef-10*, *lef-12*, and *host cell specific factor 1*), but others are conserved in all completely sequenced lepidopteran baculoviruses (*lef-6*, *lef-11*, *pk-1*, and *pp31* [*39k*]) (34, 41).

Virion structural proteins. CuniNPV contains only 8 of 17 structural protein genes conserved among lepidopteran baculoviruses (*vp39*, *vp91*, *vp1054*, *odv-ec27*, *odv-e56*, *p6.9*, *gp41*, and *p74*) (Table 1). Eleven structural genes are absent or not identifiable. These include *orf1629*, *p87*, *gp64*, *p24*, *odv-e18*, *odv-e25*, *odv-e66*, *p10*, *pp34*, *polyhedrin/granulin*, and *fp25k* (34).

Nucleocapsid proteins. CUN024 (*vp39*), CUN035 (*vp91*), and CUN008 (*vp1054*) are homologues of lepidopteran baculovirus genes encoding capsid-associated proteins. CUN024 contains codons for only two of the eight cysteine residues that are conserved in the vp39 capsid protein. CUN035 resembles the gene encoding a protein found in both the capsid and envelope of ODVs (58). CUN008 (*vp1054*) resembles the gene for a virion-associated protein that functions in nucleocapsid formation (27). Homologues of the capsid-associated proteins p87, p24, and orf1629, an essential protein associated with the basal structure of the capsid, are absent or not identifiable in CuniNPV (27).

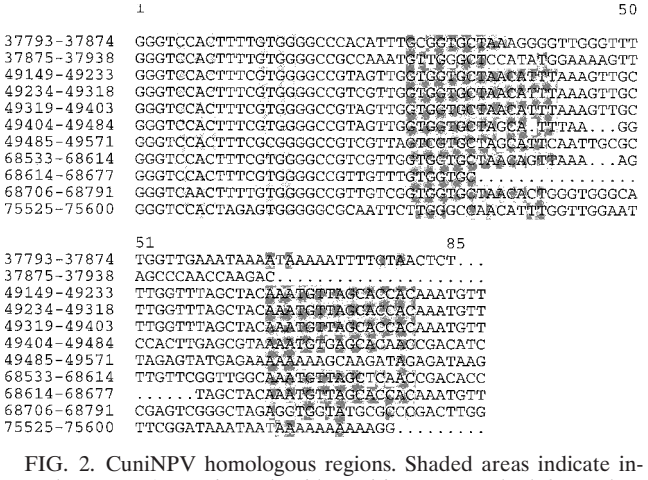

FIG. 2. CuniNPV homologous regions. Shaded areas indicate inverted repeats. Genomic nucleotide positions are on the left. Nucleotide positions in repeats are at the top. Dots represent gaps introduced by the multiple alignment program Pileup (19).

BV proteins. CuniNPV lacks a homologue of Ac*M*NPV gp64, but it does encode a homologue (CUN104) of the Ld*M*NPV envelope protein Ld130. The CUN104 product also demonstrates relatedness to Se8, the envelope fusion protein of *Spodoptera exigua* NPV (Se*M*NPV) (37), and CG4715, a *Drosophila* gene product related to Ld130 (56) (Table 1). Lepidopteran baculoviruses can be divided into two groups based on the envelope fusion proteins of their budded viruses (47). Ac*M*NPV and its close relatives utilize gp64, which is a low-pH-activated envelope fusion protein (9). In contrast, many other diverse baculoviruses, including both GVs and NPVs, lack homologues of gp64 but encode proteins related to Ld130, the envelope fusion protein of Ld*M*NPV.

Proteins associated with the occluded virus. CUN032, CUN074, and CUN102 are homologues of the genes for three proteins associated with the occluded viral envelope, and CUN033 is the homologue of a tegument protein. CUN032 is the homologue of *odv-ec27*, which encodes a protein present in ODV nucleocapsids and envelopes and may function as a cyclin (7). CUN074 is similar to the gene for p74, an ODV protein required for oral infectivity. CUN102 is the homologue of *odv-e56*, which encodes a protein associated with both virus-induced intranuclear vesicles and envelopes. CUN033 is the homologue of the gene for the ODV tegument protein gp41 (27). gp41 is required for egress of nucleocapsids from the nucleus in the pathway of budded virus synthesis (44).

The absence of several baculovirus ODV gene homologues suggests involvement of another set of viral or perhaps cellular proteins in CuniNPV ODV assembly. *odv-e25*, *odv-e66*, *and odv-e18* are absent in CuniNPV. In lepidopteran baculoviruses there are four highly conserved ODV envelope proteins (ODV-E18, ODV-E25, ODV-E56, and ODV-E66). ODV-E25 and ODV-E66 are found in the intranuclear microvesicles that are believed to be viral envelope precursors and are necessary for the morphogenesis of preoccluded virions. In addition, CuniNPV lacks a p24 homologue (Ac*M*NPV ORF 128), a protein associated with ODV and BV. Lepidopteran ODVs enter the brush border microvilli of midgut epithelial cells by fusion of the viral envelope with the cellular plasma membrane (69). The lack of ODV proteins suggests that other CuniNPV proteins may perform these cell entry functions.

TABLE 2. Comparison between CuniNPV and conserved lepidopteran genes[a]

Gene function	Gene(s) present in CuniNPV	Genes absent in CuniNPV
Transcription	*p47, lef-8, lef-9, vlf-1, lef-4, lef-5*	*lef-6, pp31 (39k), lef-11*
Replication	*lef-2, lef-1, dna-pol, helicase-1*	*lef-3, ie-1*
Structural proteins	*vp1054, gp41, vp39, vp91 p6.9, p74, odv-ec27, odv-e56*	*polyhedrin/granulin, pk-1, p10, odv-e18, odv-e25, odv-e66*
Host metabolism	*p35*	*fgf, iap, sod, ubiquitin*
Other	*alk-exo*	*dbp, fp, ie-0*
Unknown (Ac*M*NPV ORF)	023, 068, 081, 096, 098, 109, 115	013, 022, 029, 038, 053, 066, 071, 075, 076, 078, 082, 092, 093, 101, 102, 103, 106, 107, 110, 119, 139, 142, 145, 146, 150

[a] Conserved genes correspond to the genomes of seven completely sequenced baculoviruses (34).

Proteins involved with virion occlusion. CuniNPV OBs are globular, lack a polyhedron envelope and calyx structure, and typically contain about four individually enveloped virions (42). In addition, although they appear to be composed of a peptide similar in size to lepidopteran polyhedrin or granulin (about 30 kDa), amino-terminal amino acid sequence analysis did not reveal homology to other baculovirus OB proteins (42). However, the sequence did match positions 693 to 709 of CUN085, an ORF with no homology to any other known baculovirus gene. The large size of the CUN085 product (882 amino acids) suggests that it is cleaved to produce components of CuniNPV OBs. In addition, no homologues of polyhedrin or granulin genes were identified in the genome sequence.

CuniNPV also lack homologues of the polyhedron-associated proteins p10, fp25K (a conserved protein involved in polyhedra formation), and pp34, the polyhedron envelope and calyx protein (27, 34). The lack of a *pp34* homologue is reflected in the absence of a polyhedron envelope and calyx surrounding the OB. The small size of the CuniNPV OBs, the limited number of virions occluded, and the lack of an envelope and calyx structure are reminiscent of the structure of the OBs of GVs.

The absence of a *p10* gene in the CuniNPV genome is supported by observed morphological differences from cells infected with lepidopteran NPVs (42). In infected lepidopteran cells, p10 is expressed as an abundant protein (69) that is associated with nuclear and cytoplasmic fibrillar bodies during terminal stages of infection. Fibrillar material produced during CuniNPV infection, however, does not resemble fibrillar bodies associated with lepidopteran baculovirus infections. Most notably, the cytoplasmic features of CuniNPV-infected cells are microtubule bundles and irregular cisternae of smooth endoplasmic reticulum.

***bro* gene family.** The CuniNPV genome contains six baculovirus repeated ORFs (*bro* ORFs) (CUN001, CUN004, CUN005, CUN095, CUN108, and CUN109). CuniNPV *bro* ORFs are variable in length (290 to 601 amino acids) and similarity (23 to 31% amino acid identities) to other baculovirus *bro* ORFs (Table 1). The closest baculovirus homologues are the genes for *X. c-nigrum* GV ORF60 and ORF131, followed by Ld*M*NPV group III *bro d*, *c*, and *i* (39). All six CuniNPV *bro* ORFs contain the sequence for the 41-amino-acid motif common to all *bro* proteins (39) and two additional amino acid motifs conserved in group I and III *bro* proteins. Baculovirus repeated ORFs are present in 1 to 17 copies in most NPVs and GVs. Although their function is unknown, some *bro* proteins have been shown to bind DNA and may be involved in viral DNA replication (76).

Genes that may inhibit apoptosis. CuniNPV contains one gene (CUN075) with homology to the baculovirus antiapoptosis *p35* gene (16), but it lacks homologues of the *iap* (inhibitor of apoptosis) family of genes (18). Although up to four copies of *iap* genes may be present in baculovirus genomes (39), CuniNPV lacks members of this gene family. However, CUN075 demonstrates homology to *p35* genes, suggesting that it may be utilized to block apoptosis. The lack of the sequence for the 110-amino-acid carboxy-terminal region, which in Ac*M*NPV p35 mediates antiapoptotic activity (8, 13), in CUN075 is surprising and suggests a different mode of action for this gene.

Auxiliary genes. Auxiliary genes are nonessential for viral replication in cell culture, although they likely provide selective advantages in insects. CuniNPV lacks homologues of at least 14 baculovirus auxiliary genes, including those encoding superoxide dismutase, ubiquitin, inhibitor of apoptosis, protein kinase 1, and viral enhancing factors (45).

CUN039 shows relatedness to genes for insulin binding proteins of *Spodoptera frugiperda* and may belong to the auxiliary gene category. It is also related to a *Caenorhabditis elegans* gene of unknown function and *Drosophila* IMP-L2 (28, 46, 61). Insulin and related peptides are very important hormones for the regulation of growth and metabolism. The insulin-related peptide binding protein secreted from *S. frugiperda* cells is composed of two immunoglobulin-like C2 domains, binds human insulin, and inhibits insulin signaling through the insulin receptor (61). The closest homologue of the *S. frugiperda* binding protein is the essential protein IMP-L2, found in *Drosophila melanogaster*. IMP-L2 also binds insulin and related peptides (28, 46, 61) and is implicated in neural and ectodermal development (28, 46). CUN039 may have a similar role in modifying host metabolism and/or development.

Phylogeny of CuniNPV. Although clearly related to lepidopteran baculoviruses, the data from our sequence analyses indicate a number of major differences. These include low levels of identity between homologous ORFs, lack of conservation in gene order, absence of many genes present in all lepidopteran baculovirus genomes, and the lack of a homologue to polyhedrin or granulin, which are two of the most conserved baculovirus gene products (Table 2). These differences resemble those observed among viral subfamilies (1, 2) and suggest that the evolutionary distance between CuniNPV and lepidopteran baculoviruses is greater than the distance separating GVs from

NPVs. Our previous phylogenetic analyses using DNA polymerase and p74 sequences suggested that CuniNPV is a member of a baculovirus lineage distinct from lepidopteran NPVs and GVs (43). Phylogenetic trees and distance analysis of highly conserved CuniNPV genes (CUN038 and CUN074) reveals a rate of amino acid change similar to that of *Nudiviruses*, a group that has recently been excluded from the *Baculoviridae* (data not shown).

It has been suggested that some viruses (62), including baculoviruses (54, 57), may coevolve with their hosts in a process called host-dependent evolution. Molecular evidence indicates that the ancestral Lepidoptera and Diptera separated about 280 million years ago (12). The extent of the differences between the lepidopteran baculoviruses and CuniNPV may reflect this ancient separation.

ACKNOWLEDGMENTS

We thank G. F. Rohrmann for his many helpful comments and suggestions on the manuscript and A. Ciupryk and A. Zsak for excellent technical assistance.

REFERENCES

1. **Afonso, C. A., E. R. Tulman, Z. Lu, E. Oma, G. F. Kutish, and D. L. Rock.** 1999. The genome of *Melanoplus sanguinipes* entomopoxvirus. J. Virol. **73:** 533–552.
2. **Afonso, C. L., E. R. Tulman, Z. Lu, L. Zsak, G. F. Kutish, and D. L. Rock.** 2000. The genome of fowlpox virus. J. Virol. **74:**3815–3831.
3. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
4. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
5. **Barrett, J. W., H. A. Lauzon, P. S. Mercuri, P. J. Krell, S. S. Sohi, and B. M. Arif.** 1996. The putative LEF-1 proteins from two distinct *Choristoneura fumiferana* multiple nucleopolyhedroviruses share domain homology to eukaryotic primases. Virus Genes **13:**229–237.
6. **Becnel, J. J., S. E. White, B. A. Moser, T. Fukuda, M. J. Rotstein, A. H. Undeen, and A. Cockburn.** 2001. Epizootiology and transmission of a newly discovered baculovirus from the mosquitoes *Culex nigripalpus* and *C. quinquefasciatus*. J. Gen. Virol. **82:**275–282.
7. **Belyavskyi, M., S. C. Braunagel, and M. D. Summers.** 1998. The structural protein ODV-EC27 of *Autographa californica* nucleopolyhedrovirus is a multifunctional viral cyclin. Proc. Natl. Acad. Sci. USA **95:**11205–11210.
8. **Bertin, J., S. M. Mendrysa, D. J. LaCount, S. Gaur, J. F. Krebs, R. C. Armstrong, K. J. Tomaselli, and P. D. Friesen.** 1996. Apoptotic suppression by baculovirus P35 involves cleavage by and inhibition of a virus-induced CED-3/ICE-like protease. J. Virol. **70:**6251–6259.
9. **Blissard, G. W., and J. R. Wenz.** 1992. Baculovirus gp64 envelope glycoprotein is sufficient to mediate pH-dependent membrane fusion. J. Virol. **66:**6829–6835.
10. **Brendel, V., P. Bucher, I. R. Nourbakhsh, B. E. Blaisdell, and S. Karlin.** 1992. Methods and algorithms for statistical analysis of protein sequences. Proc. Natl. Acad. Sci. USA **89:**2002–2006.
11. **Burks, C.** 1999. Molecular biology database list. Nucleic Acids Res. **27:**1–9.
12. **Burmester, T., H. C. Massey, Jr., S. Zakharkin, and H. Benes.** 1998. The evolution of hexamerins and the phylogeny of insects. J. Mol. Evol. **47:**93–108.
13. **Cartier, J. L., P. A. Hershberger, and P. D. Friesen.** 1994. Suppression of apoptosis in insect cells stably transfected with baculovirus p35: dominant interference by N-terminal sequences p35(1–76). J. Virol. **68:**7728–7737.
14. **Chen, X., W. F. Ijkel, R. Tarchini, X. Sun, H. Sandbrink, H. Wang, S. Peters, D. Zuidema, R. K. Lankhorst, J. M. Vlak, and Z. Hu.** 2001. The sequence of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus genome. J. Gen. Virol. **82:**241–257.
15. **Clark, T. B., H. C. Chapman, and T. Fukuda.** 1969. Nuclear-polyhedrosis and cytoplasmic-polyhedrosis virus infections in Louisiana mosquitoes. J. Invertebr. Pathol. **14:**284–286.
16. **Clem, R. J., M. Fechheimer, and L. K. Miller.** 1991. Prevention of apoptosis by a baculovirus gene during infection of insect cells. Science **254:**1388–1390.
17. **Croizier, G., L. Croizier, O. Argaud, and D. Poudevigne.** 1994. Extension of *Autographa californica* nuclear polyhedrosis virus host range by interspecific replacement of a short DNA sequence in the p143 helicase gene. Proc. Natl. Acad. Sci. USA **91:**48–52.
18. **Crook, N. E., R. J. Clem, and L. K. Miller.** 1993. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. J. Virol. **67:**2168–2174.
19. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
20. **Eddy, S. R., G. Mitchison, and R. Durbin.** 1995. Maximum discrimination hidden Markov models of sequence consensus. J. Comput. Biol. **2:**9–23.
21. **Ewing, B., and P. Green.** 1998. Base-calling of automated sequencer traces using *Phred*. II. Error probabilities. Genome Res. **8:**186–194.
22. **Ewing, B., L. Hillier, M. C. Wendl, and P. Green.** 1998. Base-calling of automated sequencer traces using *Phred*. I. Accuracy assessment. Genome Res. **8:**175–185.
23. **Federici, B. A.** 1985. Viral pathogens of mosquito larvae. Bull. Am. Mosquito Control Assoc. **6:**62–74.
24. **Federici, B. A.** 1980. Mosquito baculovirus: sequence of morphogenesis and ultrastructure of the virion. Virology **100:**1–9.
25. **Federici, B. A., and R. E. Lowe.** 1972. Studies on the pathology of a baculovirus in *Aedes triseriatus*. J. Invertebr. Pathol. **20:**14–21.
26. **Friesen, P. D.** 1997. Regulation of baculovirus early gene expression, p. 141–170. *In* L. K. Miller (ed.), The baculoviruses. Plenum Press, New York, N.Y.
27. **Funk, C. J., S. C. Braunagel, and G. F. Rohrmann.** 1997. Baculovirus structure, p. 7–32. *In* L. K. Miller (ed.), The baculoviruses. Plenum Press, New York, N.Y.
28. **Garbe, J. C., E. Yang, and J. W. Fristrom.** 1993. IMP-L2: an essential secreted immunoglobulin family member implicated in neural and ectodermal development in *Drosophila*. Development **119:**1237–1250.
29. **Gordon, D., C. Abajian, and P. Green.** 1998. *Consed*: a graphical tool for sequence finishing. Genome Res. **8:**195–202.
30. **Guarino, L., and M. D. Summers.** 1986. Interspersed homologous DNA of *Autographa californica* nuclear polyhedrosis virus enhances delayed-early gene expression. J. Virol. **60:**215–223.
31. **Guarino, L. A., B. Xu, J. Jin, and W. Dong.** 1998. A virus-encoded RNA polymerase purified from baculovirus-infected cells. J. Virol. **72:**7985–7991.
32. **Hashimoto, Y., T. Hayakawa, Y. Ueno, T. Fujita, Y. Sano, and T. Matsumoto.** 2000. Sequence analysis of the *Plutella xylostella* granulovirus genome. Virology **275:**358–372.
33. **Hayakawa, T., R. Ko, K. Okano, S.-I. Seong, C. Goto, and S. Maeda.** 1999. Sequence analysis of the *Xestia c-nigrum* granulovirus genome. Virology **262:**277–297.
34. **Hayakawa, T., G. F. Rohrmann, and Y. Hashimoto.** 2000. Patterns of genome organization and content in lepidopteran baculoviruses. Virology **278:** 1–12.
35. **Heldens, J. G. M., Y. Liu, D. Zuidema, R. W. Goldbach, and J. M. Vlak.** 1997. Characterization of a putative *Spodoptera exigua* multicapsid nucleopolyhedrovirus helicase gene. J. Gen. Virol. **78:**3101–3114.
36. **Huang, X., and A. Madan.** 1999. CAP3: a DNA sequence assembly program. Genome Res. **9:**868–877.
37. **IJkel, W. F. J., M. Westenberg, R. W. Goldbach, G. W. Blissard, J. M. Vlak, and D. Zuidema.** 2000. A novel baculovirus fusion envelope protein with a proprotein convertase cleavage site. Virology **275:**30–41.
38. **Jones, D. T., W. R. Taylor, and J. M. Thornton.** 1994. A model recognition approach to the prediction of all-helical membrane protein structure and topology. Biochemistry **33:**3038–3049.
39. **Kuzio, J., M. N. Pearson, S. H. Harwood, C. J. Funk, J. T. Evans, J. M. Slavicek, and G. F. Rohrmann.** 1999. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology **253:**17–34.
40. **Lu, A., P. J. Krell, J. M. Vlak, and G. F. Rohrmann.** 1997. Baculovirus DNA replication, p. 171–191. *In* L. K. Miller (ed.), The baculoviruses. Plenum Press, New York, N.Y.
41. **Lu, A., and L. K. Miller.** 1997. Regulation of baculovirus late and very late gene expression, p. 193–216. *In* L. K. Miller (ed.), The baculoviruses. Plenum Press, New York, N.Y.
42. **Moser, B. A., J. J. Becnel, S. E. White, C. L. Afonso, G. F. Kutish, S. Shanker, and E. Almira.** 2001. Morphological and molecular evidence that *Culex nigripalpus* baculovirus is an unusual member of the family *Baculoviridae*. J. Gen. Virol. **82:**283–297.
43. **Nakai, K., and P. Horton.** 1999. PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization. Trends Biochem. Sci. **24:**34–36.
44. **Olszewski, J., and L. K. Miller.** 1997. A role for baculovirus GP41 in budded virus production. Virology **233:**292–301.
45. **O'Reilly, D. R.** 1997. Auxiliary genes of baculoviruses, p. 267–300. *In* L. K. Miller (ed.), The baculoviruses. Plenum Press, New York, N.Y.
46. **Osterbur, D. L., D. K. Fristrom, J. E. Natzle, S. J. Tojo, and J. W. Fristrom.** 1988. Genes expressed during imaginal disc morphogenesis: IMP-L2, a gene expressed during imaginal disc and imaginal histoblast morphogenesis. Dev. Biol. **129:**439–448.
47. **Pearson, M. N., C. Groten, and G. F. Rohrmann.** 2000. Identification of the *Lymantria dispar* nucleopolyhedrovirus envelope fusion protein provides evidence for a phylogenetic division of the *Baculoviridae*. J. Virol. **74:**6126–6131.
48. **Pearson, M. N., and G. F. Rohrmann.** 1995. *Lymantria dispar* nuclear polyhedrosis virus homologous regions: characterization of their ability to func-

tion as replication origins. J. Virol. **69:**213–321.
49. **Pearson, W. R.** 1990. Rapid and sensitive sequence comparison with FASTP and FASTA. Methods Enzymol. **183:**63–98.
50. **Possee, R. D., and G. F. Rohrmann.** 1997. Baculovirus genome organization and evolution, p. 109–140. *In* L. K. Miller (ed.), The baculoviruses. Plenum Press, New York, N.Y.
51. **Que, Q., Y. Zhang, M. Nelson, S. Ropp, D. E. Burbank, and J. L. Van Etten.** 1997. Chlorella virus SC-1A encodes at least five functional and one nonfunctional DNA methyltransferases. Gene **190:**237–244.
52. **Rapp, J. C., J. A. Wilson, and L. K. Miller.** 1998. Nineteen baculovirus open reading frames, including LEF-12, support late gene expression. J. Virol. **72:**10197–10206.
53. **Rasmussen, C., D. J. Leisy, P. S. Ho, and G. F. Rohrmann.** 1996. Structure-function analysis of the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus homologous region palindromes. Virology **224:**235–245.
54. **Rohrmann, G. F.** 1986. Evolution of occluded baculoviruses, vol. 1. CRC Press, Boca Raton, Fla.
55. **Rohrmann, G. F.** 1999. Nucleopolyhedrovirus, 2nd ed. Academic Press, London, United Kingdom.
56. **Rohrmann, G. F., and P. A. Karplus.** 2001. Relatedness of baculovirus and gypsy retrotransposon envelope proteins. BMC Evol. Biol. **1:**1–9.
57. **Rohrmann, G. F., M. N. Pearson, T. J. Bailey, R. R. Becker, and G. S. Beaudreau.** 1981. N-terminal polyhedrin sequences and occluded baculovirus evolution. J. Mol. Evol. **17:**329–333.
58. **Russell, R. L., and G. F. Rohrmann.** 1997. Characterization of P91, a protein associated with virions of an Orgyia pseudotsugata baculovirus. Virology **233:**210–223.
59. **Salzberg, S. L., A. L. Delcher, S. Kasif, and O. White.** 1998. Microbial gene identification using interpolated Markov models. Nucleic Acids Res. **26:**544–548.
60. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
61. **Sloth Andersen, A., P. Hertz Hansen, L. Schaffer, and C. Kristensen.** 2000. A new secreted insect protein belonging to the immunoglobulin superfamily binds insulin and related peptides and inhibits their activities. J. Biol. Chem. **275:**16948–16953.
62. **Soeda, E., T. Maruyama, J. Arrand, and B. Griffin.** 1980. Host-dependent evolution of three papova viruses. Nature **285:**165–167.
63. **Sonnhammer, E. L. L., S. R. Eddy, E. Birney, A. Bateman, and R. Durbin.** 1998. Pfam: multiple sequence alignments and HMM-profiles of protein domains. Nucleic Acids Res. **26:**320–322.
64. **Staden, A., and A. D. McLachlan.** 1982. Codon preference and its use in identifying protein coding regions in long DNA sequences. Nucleic Acids Res. **10:**141–156.
65. **Staden, R.** 1982. An interactive graphics program for comparing and aligning nucleic acid and amino acid sequences. Nucleic Acids Res. **10:**2951–2961.
66. **Stiles, B., P. E. Dunn, and J. D. Paschke.** 1983. Histopathology of a nuclear polyhedrosis infection in *Aedes epactius* with observations in four additional mosquito species. J. Invertebr. Pathol. **41:**191–202.
67. **Sutton, G. G., O. White, M. D. Adams, and A. R. Kerlavage.** 1995. TIGR assembler: a new tool for assembling large shotgun sequencing projects. Genome Sci. Technol. **1:**9–19.
68. **Tatusov, R. L., S. F. Altschul, and E. V. Koonin.** 1994. Detection of conserved segments in proteins: iterative scanning of sequence databases with alignment blocks. Proc. Natl. Acad. Sci. USA **91:**12091–12095.
69. **Williams, G. V., and P. Faulkner.** 1997. Cytological changes and viral morphogenesis during baculovirus infection, p. 61–107. *In* L. K. Miller (ed.), The baculoviruses. Plenum Press, New York, N.Y.
70. **Winstanley, D., and D. O'Reilly.** 1999. Granuloviruses, 2nd ed. Academic Press, London, United Kingdom.
71. **Wu, Y., and E. B. Carstens.** 1998. A baculovirus single-stranded DNA binding protein, LEF-3, mediates the nuclear localization of the putative helicase P143. Virology **247:**32–40.
72. **Yang, S., and L. K. Miller.** 1999. Activation of baculovirus very late promoters by interaction with very late factor 1. J. Virol. **73:**3404–3409.
73. **Yang, S., and L. K. Miller.** 1998. Control of baculovirus polyhedrin gene expression by very late factor 1. Virology **248:**131–138.
74. **Yang, S., and L. K. Miller.** 1998. Expression and mutational analysis of the baculovirus very late factor 1 (*vlf-1*) gene. Virology **245:**99–109.
75. **Zanotto, P. M., B. D. Kessing, and J. E. Maruniak.** 1993. Phylogenetic interrelationships among baculoviruses: evolutionary rates and host associations. J. Invertebr. Pathol. **62:**147–164.
76. **Zemskov, E. A., W. Kang, and S. Maeda.** 2000. Evidence for nucleic acid binding ability and nucleosome association of *Bombyx mori* nucleopolyhedrovirus BRO proteins. J. Virol. **74:**6784–6789.