Genomic Sequence of *Spodoptera frugiperda* Ascovirus 1a, an Enveloped, Double-Stranded DNA Insect Virus That Manipulates Apoptosis for Viral Reproduction[▽]

Dennis K. Bideshi,¹ Marie-Véronique Demattei,² Florence Rouleux-Bonnin,² Karine Stasiak,² Yeping Tan,¹ Sylvie Bigot,² Yves Bigot,² and Brian A. Federici^{1,3*}

Department of Entomology, University of California, Riverside, California 92521¹; Unit of Eukaryotic Parasite Genetics, FRE CNRS 2969, University François Rabelais of Tours, Tours, France²; and Interdepartmental Graduate Programs in Genetics, Microbiology, and Cell, Molecular, and Developmental Biology, University of California, Riverside, California 92521³

Received 1 August 2006/Accepted 8 September 2006

Ascoviruses (family *Ascoviridae*) are double-stranded DNA viruses with circular genomes that attack lepidopterans, where they produce large, enveloped virions, 150 by 400 nm, and cause a chronic, fatal disease with a cytopathology resembling that of apoptosis. After infection, host cell DNA is degraded, the nucleus fragments, and the cell then cleaves into large virion-containing vesicles. These vesicles and virions circulate in the hemolymph, where they are acquired by parasitic wasps during oviposition and subsequently transmitted to new hosts. To develop a better understanding of ascovirus biology, we sequenced the genome of the type species *Spodoptera frugiperda* ascovirus 1a (SfAV-1a). The genome consisted of 156,922 bp, with a G+C ratio of 49.2%, and contained 123 putative open reading frames coding for a variety of enzymes and virion structural proteins, of which tentative functions were assigned to 44. Among the most interesting enzymes, due to their potential role in apoptosis and viral vesicle formation, were a caspase, a cathepsin B, several kinases, E3 ubiquitin ligases, and especially several enzymes involved in lipid metabolism, including a fatty acid elongase, a sphingomyelinase, a phosphate acyltransferase, and a patatin-like phospholipase. Comparison of SfAV-1a proteins with those of other viruses showed that 10% were orthologs of *Chilo* iridescent virus proteins, the highest correspondence with any virus, providing further evidence that ascoviruses evolved from a lepidopteran iridovirus. The SfAV-1a genome sequence will facilitate the determination of how ascoviruses manipulate apoptosis to generate the novel virion-containing vesicles characteristic of these viruses and enable study of their origin and evolution.

The family *Ascoviridae* was erected recently to accommodate several new species of large double-stranded DNA (dsDNA) viruses with circular genomes that attack insects of the order *Lepidoptera* at the larval and pupal stages, causing a chronic, fatal disease (38). Viruses of this family are characterized by large, enveloped virions with a distinctive reticulate surface pattern. Depending on the species, virions are either bacilli-form or allantoid (sausage shaped), contain an internal lipid membrane surrounding the DNA/protein core, and are composed of at least 12 structural proteins, ranging in mass from 10 to 200 kDa (40).

These structural characteristics of the virions are sufficient to distinguish ascoviruses from all other large dsDNA viruses. However, the most novel feature of ascoviruses is not their virion structure, but rather their unusual cellular pathology and transmission. Unlike for all other viruses, a variety of evidence suggests that ascoviruses induce apoptosis as part of a mechanism that enhances their reproduction and transmission. A typical pattern of cytopathology, as exemplified by *Spodoptera frugiperda* ascovirus 1a (SfAV-1a), the type species, begins with nuclear hypertrophy and cleavage of host DNA, followed by lysis of the nucleus and fragmentation of the nu-

clear membrane. Recent studies demonstrating that SfAV-1a synthesizes an executioner caspase provide evidence that this virus plays a direct role in initiating apoptosis (16). After nuclear lysis, cellular hypertrophy ensues and what appear to be destined to become apoptotic bodies begin to form at the cell periphery by membrane invagination. However, rather than degenerate, the developing apoptotic bodies are rescued by the virus and go on to form large virion-containing vesicles, also referred to as viral vesicles, that typically range from 5 to 10 μ m in diameter. These disseminate to the hemolymph, where they circulate for weeks, until the infected larva or pupa dies (39).

Ascoviruses are very poorly infectious per os, an unusual trait for insect viruses other than iridoviruses (family *Iridoviridae*). Thus, circulation of viral vesicles in the hemolymph apparently evolved to facilitate transmission. Several field and laboratory studies have shown that endoparasitic wasps acquire ascovirus virions and viral vesicles on their ovipositor while probing infected larvae or pupae during egg-laying. Contamination of the ovipositor results in very high levels of infection, typically greater than 80%, in larvae subsequently probed by these wasps. That this mechanism of transmission operates under field conditions is supported by studies showing that ascovirus isolates can be obtained by allowing field-collected wasps to probe laboratory-reared larvae (46, 103).

Whereas field studies of ascoviruses indicate that they occur commonly, particularly in populations of lepidopteran species

* Corresponding author. Mailing address: Department of Entomology, University of California—Riverside, Riverside, CA 92521. Phone: (951) 827-5006. Fax: (951) 827-3086. E-mail: brian.federici@ucr.edu.

[▽] Published ahead of print on 20 September 2006.

belonging to the family *Noctuidae* and especially when parasitic wasp populations are high, only five ascovirus species are recognized currently (37). In addition to SfAV-1a, these are *Trichoplusia ni* ascovirus 2a (TnAV-2a), *Heliothis virescens* ascovirus 3a (HvAV-3a), *Diadromus pulchellus* ascovirus 4a (DpAV-4a), and *Spodoptera exigua* ascovirus 5a (SeAV5a). Of these, with the exception of DpAV-4a, which attacks the leaf moth, *Acrolepiopsis assectella* (family *Hyponomeutidae*), at the pupal stage, all known ascovirus species attack noctuids. Some ascoviruses, such as TnAV-2a and HvAV-3a, have broad host spectra, being able to replicate in species of several noctuid genera. In contrast, SfAV-1a has a narrow host range, apparently capable of replicating only in *Spodoptera* species. The most unusual ascovirus is DpAV-4a, which replicates in its wasp vector and is transmitted vertically to its lepidopteran host, in which it replicates much more extensively (17, 18).

To begin to develop a better understanding of the genetic basis of ascovirus pathogenesis, host spectra, and evolution, we sequenced the SfAV-1a genome, which we report here. Consistent with its unusual pathology, we show that this genome contains many genes unique among viruses.

MATERIALS AND METHODS

Genomic DNA library. Propagation of SfAV-1a, referred to hereafter as SfAV, and preparation of viral DNA were performed as described previously (17, 40), using a clone of this species originally propagated in *S. frugiperda* (40). Briefly, 50 µg of SfAV DNA was sheared by sonication (20 W for 2.5 min with 1-s pulses) to generate fragments ranging in size from 0.5 to 3 kbp. DNA fragments were bluntended with S1 nuclease and T4 DNA polymerase (New England Biolabs), and EcoRI linkers were ligated at both ends. Fragments of approximately 0.85 to 1.1 kbp were purified from agarose gel by using a QIAquick gel extraction kit (QIAGEN) and ligated to the EcoRI site in pUC18.

DNA sequencing. Plasmids were isolated and purified using standard protocols (8). Nucleotide sequences were determined by dideoxynucleotide sequencing (84) using a Sequitherm long-read cycle sequencing kit with universal and reverse IRD800 fluorescently labeled primers (Epicentre Technologies). DNAs were amplified by PCR (25 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 15 s, and polymerization at 70°C for 1 min). Nucleotide sequences for both strands were generated using a model 4200 DNA long-read sequencer (Li-cor). Each nucleotide was sequenced an average of eight times to verify accuracy.

Genome analyses. Genomic DNA composition, structure, and codon usage were analyzed using several desktop and online programs (DNASTAR [Lasergene], http://www.info.univ-angers.fr/pub/gh/signets_gh.htm, and the <http://www.kazusa.or.jp/codon/>). The SfAV genomic map was constructed using Vector NTI (Invitrogen). Nucleotide sequence and protein database searches were performed using the BLAST programs at the NCBI website (<http://www.ncbi.nlm.nih.gov>). For more-refined analyses, conserved motifs and domains and putative functions of deduced SfAV proteins composed of 49 or more amino acids with homologies to other proteins in sequence databases were identified using several online programs, as follows: for physicochemical properties of proteins, http://www.iut-arles.univ-mrs.fr/w3bb/d_abim/compo-p.html and <http://www.expasy.ch/cgi-bin/protscale.pl>; for conserved motifs and domains, http://hits.isb-sib.ch/cgi-bin/motif_scan, <http://www.expasy.org/cgi-bin/scanprosite>, http://smart.embl-heidelberg.de/smart/set_mode.cgi?GENOMIC=1, <http://www.cbs.dtu.dk/services/>, http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hmmbuild.html, <http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>, <http://supfam.org/SUPERFAMILY/>, and http://idefix.univ-rennes1.fr:8080/GenQuest/ouils.php3?id_rubrique=40; for secretion and maturation motif predictions, <http://www.cbs.dtu.dk/services/SecretomeP/> (non-classical and leaderless secretion of proteins), <http://www.cbs.dtu.dk/services/TatP-1.0/> (twin arginine signal peptides), <http://www.cbs.dtu.dk/services/SignalP/> (peptide signals), and <http://www.cbs.dtu.dk/services/TMHMM-2.0/> (transmembrane domains); for dimerization motifs, http://bmecr-www.bu.edu/bioinformatics/wd_search.html (WD domain repeat); for leucine zippers, <http://2zip.molgen.mpg.de/index.html>; for DNA-binding motifs, <http://www.sangamo.com/index.php> and <http://www.scripps.edu/mb/barbas/zfdesign/zfdesignhome.php> (zinc finger domain) and <http://subaru2.univ-lemans.fr/sciences/lbge/MLEsdatabase/TOOLS/hth.php> and http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hth.html (helix-turn-

helix motif); for posttranslational modification, <http://www.cbs.dtu.dk/services/NetPhosK/> and <http://pred.ngri.re.kr/PredPhospho.htm> (kinase-specific eukaryotic protein phosphorylation sites); for cellular location, <http://www.cbs.dtu.dk/services/TargetP/>, <http://motif.genome.jp/>, <http://www.cbs.dtu.dk/services/ProtFun/>, <http://elm.eu.org/>, <http://research.i2r.a-star.edu.sg/CysView/>, and <http://swift.cmbi.ru.nl/cyscys/web/> (subcellular location of proteins: mitochondrial, chloroplastic, secretory pathway, or other); for cellular trafficking, <http://subaru2.univ-lemans.fr/sciences/lbge/MLEsdatabase/TOOLS/nls.php> and <http://cubic.bioc.columbia.edu/cgi/var/nair/resonline.pl> (nuclear localization signals) and <http://www.cbs.dtu.dk/services/NetNES/> (leucine-rich nuclear export signals in eukaryotic proteins); and for the disulfur bridge, <http://research.i2r.a-star.edu.sg/CysView/> and <http://swift.cmbi.ru.nl/cyscys/web/>.

Nucleotide sequence accession number. The nucleotide sequence of the SfAV genome has been deposited in the GenBank/EMBL/DBJ databases with the accession number (AM 398843).

RESULTS AND DISCUSSION

General characteristics and coding capacity of the SfAV genome. Of the five recognized species of ascoviruses, none has been sequenced. Thus, we determined the complete genome sequence of SfAV, the ascovirus type species, by sequencing 1,600 overlapping clones, each approximately 1 kilobase pair (kbp) in length, from a shotgun library in pUC18. The SfAV genome consisted of a circular dsDNA molecule of 156,922 bp, with a G+C content of 49.26%.

Other than ascoviruses, several types of large dsDNA viruses are known to attack insects. The most common of these are the baculoviruses (family *Baculoviridae*), iridoviruses (family *Iridoviridae*), and entomopoxviruses (family *Poxviridae*). Of these, based on molecular phylogenetic data, the ascoviruses are most closely related to the iridoviruses and appear to have evolved from a lepidopteran iridovirus (98, 99). Interestingly, however, the genome size and G+C content of SfAV differed significantly from those of the *Chilo* iridescent virus (CIV), the only lepidopteran iridovirus sequenced to date. The CIV genome is 212 kbp, with a G+C content of 28.63%. Of nine other iridoviruses for which the genome sequence is available, eight of these are smaller (98 to 140 kbp) than SfAV, whereas the *Lymphocystivirus* iridovirus (lymphocystis disease virus isolated in China) has a larger genome (186 kbp). The G+C contents of these viruses differ widely, ranging from 27.25 to 54.8% (72). Comparison to other dsDNA viruses of insects shows that the genome size of SfAV is larger than those of most baculoviruses. These range in size from approximately 81 kbp to 178 kbp, with G+C contents ranging from 32.4% to 50.9% (61). With respect to insect poxviruses, these have larger genomes and lower G+C ratios than SfAV. The grasshopper entomopoxvirus of *Melanoplus sanguinipes*, for example, is 236 kbp, with a G+C ratio of 18.3%, and the lepidopteran entomopoxvirus from *Amsacta moorei* is 232 kbp, with a G+C content of 17.8% (3, 12). Whereas ascoviruses contain some genes related to those of baculoviruses and nudiviruses, molecular phylogenetic analyses of replication enzymes and structural proteins (for example, DNA polymerase and major capsid protein genes, respectively) indicate that they are much more closely related to the large dsDNA viruses that initiate replication in the cytoplasm, viruses such as *Chilo* IV and the phycodnaviruses (97, 98).

In regard to other general characteristics of the SfAV genome, two large repeat sequences of 2.9 kbp, each with inverted terminal repeats (ITRs), were found head-to-head be-

FIG. 1. Schematic illustration of the organization of the *Spodoptera frugiperda ascovirus 1a* genome. Predicted ORFs are indicated by their locations, orientations, and putative sizes. White arrows represent ORFs in the forward strand, whereas gray arrows identify those in the complement strand. The relative positions of the 2.9-kbp fragments, organized as inverted repeats at one locus spanning the ~80-kbp to 94-kbp positions and as a 5'- and 3'-truncated repeat at a second locus spanning the ~147.5-kbp to 149.5-kbp positions, are also shown.

tween the ~80-kbp and 94-kbp positions (Fig. 1). A fragment of the 5'- and 3'-truncated 2.9-kbp repeated sequence is also present in the region spanning the ~147.5-kbp to 149.5-kbp positions. These ITRs may be delineating regions in the SfAV genome where reciprocal changes of configurations (linear versus circular) might occur during the replication. This possibility is based on prior observations that these ITRs can differ in size, being smaller in two variants, SfAV-1b and SfAV-1c (19), which suggested that only a portion of these large repeats may be essential for this virus.

For the purpose of orientating the open reading frames (ORFs) contained in the SfAV genome, the A in the translation start codon (ATG) of the DNA polymerase gene was designated the first nucleotide position. Computer-assisted analyses of the SfAV genome revealed 123 ORFs, which were predicted to code for proteins containing 65 to 1,157 amino acid residues containing conserved domains and motifs (Fig. 1 and Table 1). Of these, only three (44, 53, and 98) completely overlapped with ORFs in the complementary strand (Fig. 1). In addition, 67 other putative ORFs, typically small, encoding

TABLE 1. Properties of ORFs within the SfAV-1a genome^a

ORF	Nucleotide positions in genome	No. of amino acids	Molecular mass (kDa)	Putative function of encoded peptide	Conserved motifs and domains ^b	Closest viral or cellular homologue ^c
001	1 > 3315	1,104	125.8	DNA polymerase B	PS00116 DNA polymerase B, ^d PS00778 acid phosphatase active site, ^d POLBc, DNA polymerase type B family (232-629, 73.8%), ^{d,e} DNA pol B elongation subunit (713-1072, 39.4%), ^{d,e} One transmembrane helix at the C terminus	Q9QSK2 ORF37L CIV
002	4222 < 4602	126	13.7	Cytochrome oxidase assembly factor	One transmembrane anchor helix	BAD65436 <i>Bacillus clausii</i>
003	4895 < 5581	228	25.4		RNA_pol_A-N term (233-528, 99.3%), ^e RNA_pol_Rpb1_3 (514-661, 92.5%), ^e RNA_pol_Rpb1_4 (690-790, 79.1%), ^e RpoC beta/160-kDa subunit (24-862, 97.3%), ^e DEXDe/DEHX box helicase (101-218, 82.6%), ^e SNF2 family N terminus (102-348, 80.6%), ^e HepA superfamily II DNA/RNA, SNF2 family (109-360, 29.4%), ^e SrmB DNA/RNA helicases (103-211, 22.4%), ^e One transmembrane helix at the C terminus	AAF48900 <i>Drosophila melanogaster</i> CAC37036 <i>Amycolatopsis lactamidurans</i> NP 689299 ORF 94 <i>Mamestra configurata</i> NPV
004	5620 > 6324	234	26.1		One transmembrane anchor helix	O55766 ORF 176R CIV
005	6704 > 7312	202	23.7		One transmembrane anchor helix	O9QSK4 ORF 22L CIV
006	7330 > 7959	209	24.2	DNA-directed RNA polymerase subunit C	One transmembrane anchor helix	
007	7972 > 8634	220	24.4		One transmembrane anchor helix	
008	9257 < 11914	885	98.4		One transmembrane anchor helix	
009	12281 > 15217	978	110.8	DEAD-like helicase of the SNF2 family	One transmembrane helix at the C terminus Alpha/beta-hydrolase family ^{d,e,f} , one transmembrane helix at the N terminus, one membrane anchor signal, esterase_lipase (144-265, 25.8%), ^e CO_esterase carboxylesterase (143-576, 85.7%), ^e PS00142 neutral zinc metalloproteinase, zinc-binding region, ^e metalloprotease (zincins), catalytic domain (71-233, 100%), ^e peptidase_M10 matrixin (87-166, 74.8%), ^e PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAF05275.1 ORF 161 <i>Xestia c-nigrum</i> GV
010	15402 > 15773	123	13.8	Lipid metabolism, juvenile hormone esterase	One transmembrane helix at the C terminus	Q761LA5 <i>Apis mellifera</i> , juvenile hormone esterase
011	15630 < 16388	252	28.2		One transmembrane helix at the C terminus	
012	16634 > 17386	250	28.2		One transmembrane helix at the C terminus	
013	17679 < 19457	592	67.3		One transmembrane helix at the C terminus	
014	19998 < 21158	386	44.5	Membrane-type matrix zinc-dependent metalloproteinase	One transmembrane helix at the C terminus	AAAB94472 ORF 165R CIV, AAM70239 <i>Phthorimaea operculella</i> GV
015	21542 > 23245	567	64.2	Inhibition of apoptosis	PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAF33613 ORF 84 <i>Spodoptera exigua</i> MNPV
016	23363 > 23695	110	12.6		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	Q6DDY3 Birc2-like protein
017	23778 > 24440	220	24.5		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAA98918 <i>Drosophila melanogaster</i> male-specific lethal protein-1
018	24498 > 25049	183	21.0		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	Q3YIMZ6 GroEL chaperonin
019	25445 > 26260	271	30.0	GroEL-like chaperonin	PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	EAL69089 <i>Dicyostelium discoideum</i>
020	26463 > 27782	439	50.0		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	
021	27665 < 27880	71	8.6		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	
022	27937 > 29190	417	47.7		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	
023	29271 > 30092	273	31.3	RNase III	PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	O55748 ORF 142R CIV
024	29844 < 30350	168	19.0		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	O55748 ORF 142R CIV
025	30100 > 30792	230	25.7		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	NP_017054 <i>Oryza sativa</i>
026	30915 < 31307	130	15.2		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	Q96ST8 inhibitor of apoptosis protein
027	31405 < 33420	671	77.2	Inhibition of apoptosis	PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	CAD24792 wild potato mosaic virus polyprotein
028	33481 > 34545	354	40.5		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	Q99176 protein SRN2 (vacuolar protein sorting-associated protein VPS37) (<i>Saccharomyces cerevisiae</i>)
029	34939 > 36126	395	45.4		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAK82143 ORF 282R CIV
030	36633 < 36926	97	11.6		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAK82143 ORF 282R CIV
031	37033 < 37350	105	12.3	Putative ligase	PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAK82143 ORF 282R CIV
032	37527 < 38480	317	37.3		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAK82143 ORF 282R CIV
033	38449 > 39165	238	27.5		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAK82143 ORF 282R CIV
034	39287 < 40981	564	65.6		PS50089 zinc finger RING type profile, ^d PEX10 RING finger containing E3, ^e ubiquitin ligase (59-102, 17.3%)	AAK82143 ORF 282R CIV

035	42596 > 43321	241	25.6	GIY-YIG-like endonuclease	One transmembrane helix at the C terminus	Q91FT0 ORF 242L CIV
036	43342 > 44061	239	26.2			NP916197.1 <i>Oryza sativa</i>
037	43964 < 44302	112	13.0			EAR81401 Ttr4Rp
038	44383 < 44973	196	22.2	Thymidine kinase	Transmembrane helices at the N and C termini ^e dNK deoxynucleoside kinase (61–206, 96.6%) ^e Tnk, thymidylate kinase (8–207, 96.6%) ^e	O55749 ORF 143R CIV
039	44987 > 45562	191	21.0		Capsid, iridovirus MCP (23–461, 98.6%) ^f	Q05815 ORF 274L CIV
040	45521 < 46153	210	24.5	Major capsid protein	One transmembrane helix at the C terminus	EAT48401.1 <i>Aedes aegypti</i>
041	46192 > 47577	461	50.9		One transmembrane helix at the C terminus	U82510 ORF 112 <i>Cydia pomonella</i> GV,
042	47651 < 48289	212	25.2	DNA metabolism, partitioning, recombination, replication and repair	Intermediate filament protein (5–243, 74.7%) ^e reovirus_sigma attachment protein (97–260, 32.7%) ^e SMC, chromosome segregation ATPase (3–432, 28%) ^e SbcC ATPase (10–368, 41.9%) ^e HEC1, interacts with SMC proteins in chromosome segregation (13–230, 34.4%) ^e PS00867 carbonoyl-phosphate synthase subdomain signature 2 PS with cut between positions 17/18	AF499596 ORF 104 <i>Phidhormiaca operculella</i> GV, Q99176 protein SRN2 (vacuolar protein sorting-associated protein VPS37) (<i>Saccharomyces cerevisiae</i>), EAR81043 Thr 2FP
043	48301 < 48987	228	26.0			
044	49974 > 50723	249	27.1			
045	50943 > 51734	263	28.8			
046	49784 < 51448	554	63.4			
047	51789 < 52538	249	28.7	DNA puffC4B-like protein		P22312 PuflII/9-2 protein
048	53484 < 55181	565	64.6			AY521625 ORF039L SGIV, AAK82240 ORF380R CIV
049	55849 < 56449:exon1 55245 < 55726; exon2 54683 < 55140:exon3			Polymorphic ORF with an allele described in AJ4370060	PS50059 zinc finger RING domain	
050	55214 < 55726	170	19.6	Homer homolog		NP_148816 ORF32 <i>Cydia pomonella</i> GV
051	55808 < 56230	140	16.4	Protein with a zinc-binding site		EAL67040 <i>Dicystotellum discoideum</i>
052	56523 > 59996	1,157	128.1	DNA-directed RNA polymerase II subunit 2	COG5236, RING/U box (56–119, 12.8%) ^e RNA_pol_Rbp2_1 (18–202, 51.1%) ^e RNA_pol_Rbp2_6 (693–1058, 100%) ^e RNA_pol_Rbp2_1 (352–442, 24.3%) ^e beta and beta-prime subunit of DNA- dependent RNA polymerase (2–1153, 99%) ^e PS with cut between positions 25/26	Q91F97 ORF 428L CIV
053	57135 < 58028	297	33.9	Myristylated membrane protein		Q91F17 ORF254L CIV
054	60049 > 60885	278	30.3		Domain metallo-dependent calcineurin-like phosphatase superfamily (sphingomyelinase phosphodiesterase, nucleotidases, nucleases) (10–191, 98.4%) ^{e,f} SbcD DNA repair exonuclease (49–286, 67.9%) ^f	Q91FR8 ORF254L CIV
055	60944 > 61882	312	35.1		Two transmembrane helices in the middle and C terminus	O55708 ORF67R CIV
056	61965 > 62996	343	37.6		FAD-dependent thiol oxidase Evr1/Alr family (25–107, 87.9%) ^{e,f} mitochondrial sulphydryl oxidase (2–107, 56.9%) ^f	EAA59097, <i>Aspergillus nidulans</i>
057	63105 < 63878	257	29.3			AAK69357 pericentriolar material 1 protein
058	63947 < 64921	324	37.0	Lipid metabolism SbcD DNA repair exonuclease		Q91F58 ORF244L CIV
059	64954 < 66051	365	41.8			
060	66345 > 66581	78	8.6	Thiol oxidoreductase-like protein		Q91FH7 ORF347L CIV
061	66951 > 67595	214	25.3		Two transmembrane helices located at N and C termini, one membrane anchor signal	
062	68065 < 68277	70	7.9			Q9ZGC4 NDP-hexose 2,3-dehydratase
063	68294 > 68647	117	13.1	Hexose 2,3-dehydratase		Q8QZQ9 ORF209R CIV
064	68856 > 71309	817	90.0	Serine/threonine-like protein kinase		Q91FD1 ORF393L CIV
065	71571 > 72551	326	37.7		XPG_XPG_N4 region (1–321, 88%) ^{e,f} HhH2 motif Rad2 family (249–316, 93.3%) ^e 5' to 3' exonuclease (165–296, 56.2%) ^{e,f} virion host shutoff protein (195–272, 16.7%) ^f	Q91FF5 ORF 369L CIV
066	72826 < 73896	356	40.8	FEN-1/FLAP-like endonuclease	RNA_pol_Rpb_5 (61–134, 13.9%, and 292–366, 14.3%) ^e RpoC beta/160-kDa subunit (67–371, 37.3%) ^f	
067	74345 > 75733	462	52.2	DNA-directed RNA polymerase II subunit 1		Q91F11 ORF 343L CIV
068	75871 > 76599	242	27.1			

Continued on following page

TABLE 1—Continued

ORF	Nucleotide positions in genome	No. of amino acids	Molecular mass (kDa)	Putative function of encoded peptide	Conserved motifs and domains ^b	Closest viral or cellular homologue ^c
069	78068 > 78526	152	17.0		Polymorphic locus with the BRO-like protein 4 allele, BRO-N Bro family (21–63, 45.4%) ^e	AAK96307 <i>Helicoverpa armigera</i> NPV BRO-A
070	78564 > 79385	273	31.0		Polymorphic locus with the BRO-like protein 5 allele, BRO-N Bro family (21–63, 45.4%) ^e	AAK84045 <i>Chrysodeixis chalcites</i> NPV BRO-D
071	80029 < 80976	315	38.0			AAK96306 <i>Helicoverpa armigera</i> NPV BRO-A
072	93888 < 94424	178	20.3			P55212 human caspase 3/7 BC078049
073	94542 > 95408	288	32.6	Proapoptotic executioner caspase		Q5VSD3 nove protein, similar to rodent cell growth regulator
074	95472 < 96224	250	27.4	Inhibition of apoptosis		O80656 senescence-associated endonuclease Y084 21.7-kDa protein in GP41-PNK intergenic region of <i>Autographa californica</i> MNPV
075	96304 > 97089	261	29.6	Nuclease	Caspase p10/p20 domains (4–277, 99.6%) ^{d,e,f}	Q8JMD4 ORF18 <i>Mamestra configurata</i> NPV-B, YR854_YL770_YL079_ and YR104_MIMIV minivirus, ORFs associated with bacterial transposases
076	97271 > 98071	266	31.0		S1/P1 nuclease (113–258, 56.3%) ^{e,f}	Q9PYT5 ORF 108 <i>Xestia c-nigrum</i> GV
077	98506 > 100326	606	70.4		C-terminal zinc finger DNA binding domain similar to that of the ISC1316, transposase 35 domain (505–586, 78.3%) ^f	AAG55803 ORF 60 <i>Helicoverpa armigera</i> NPV AAG55803 ORF 60 <i>Helicoverpa armigera</i> NPV AAY84049 ORF 118 <i>Chrysodeixis chalcites</i> NPV
078	100498 > 101178	226	25.4		PS with cut between positions 15/16	Q2TA96 eukaryotic translation factor 2 alpha kinase, AA01368 <i>Bombyx mori</i> cIF 2A kinase
079	101496 > 102587	363	40.2		BRO-like protein 11 (14–119, 85%) ^{d,e}	Q9NR97 Toll-like receptor 8
080	102839 > 103165	108	12.5		BRO-like protein 11 (14–60, 93%) ^{d,e}	Q91FM9 ORF 295L CIV
081	103331 > 103879	182	21.1	Makorin-like protein E3-ubiquitin ligase	PS50089 zinc finger RING-type (90–137, 97.8%) ^d RING/Ubox/E3 ubiquitin ligase (91–140, 62%) ^{e,f}	Q91FL7 ORF 307L CIV
082	103879 > 105240	453	52.0	Protein kinase	Serine/threonine and tyrosine protein kinase catalytic domain (1–100, 39%) ^{d,e}	
083	105249 > 106235	328	38.0	Toll-like receptor	PS with cut between positions 22/23 (83–148)	
084	107829 < 110975	1,048	119.3		Dynein-like beta chain (464–821, 41%) ^f	
085	111129 > 111518	129	14.1		One transmembrane helix in the middle region	
086	111570 < 112232	220	25.6	Putative UvrD/REP helicase: DNA topoisomerase I	Two transmembrane helices at the N terminus (90–208)	
087	113610 < 114359	249	28.8	Fatty acid elongase	ELO GNS1/SUR4 family ^e six transmembrane helices (4–232, 76.6%)	P40319 <i>Saccharomyces cerevisiae</i> elongation of fatty acid protein 3, AJ581527 ORF 49 fowlpox virus
088	114787 > 115443	218	25.0	Endoprotease		AAC64533 turkey adenovirus
089	115703 > 116038	111	13.0			EAL61802 <i>Dicystotellum discoideum</i>
090	118746 > 121526	926	104.6	Lipopolysaccharide metabolism, tyrosine protein kinase	Lipopolysaccharide-modifying enzyme (229–500, 95.7%) ^{e,f}	T03180 ORF 179R CIV
091	121613 < 121981	122	14.4	Yabby-like transcription factor	tyrosine kinase (217–489, 54%) ^{d,e}	Q91FC4 ORF 401 CIV
092	122199 > 122855	218	24.7		HMG_box (50–119, 52%) ^f	EAL85601 <i>Aspergillus fumigatus</i>
093	122909 < 123838	309	34.5	Lipid metabolism	FabD/lysophospholipase/patain-like phospholipase (27–207, 100%) ^{e,f} esterase, alpha/beta hydrolase (27–225, 60.1%) ^{e,f} one transmembrane helix at the N terminus	Q91F63 ORF 463 CIV
094	123753 > 124691	312	35.6			AAK8403 ORF 104 <i>Chrysodeixis chalcites</i> NPV
095	125523 < 126227	234	26.3	RecD exonuclease V, alpha subunit helicase	P loop containing nucleoside triphosphate hydrolase activity, ^{d,e,f} AAA superfamily of ATPases (32–159, 94.6%), viral RNA helicase superfamily (35–151, 41.3%), RecD, ATP-dependent exonuclease (exonuclease V) (34–160, 16.8%)	AAK70235 helicase-2 <i>Lymantria dispar</i> MNPV XP_782597 helitron-like helicase
096	126167 > 126955	262	29.0		One transmembrane helix at the C terminus	AAW41422 <i>Cryptococcus neoformans</i>
097	127021 > 127503	160	18.2	BRCA1-like protein	PS00518 zinc finger RING domain (98–147, 17%) ^{d,e} E3 ubiquitin signal at C terminus, one membrane anchor signal	AAK92675 BRCA1-like protein of <i>Monodelphis domestica</i>
098	128198 > 128611	137	15.0			AAK180068 ORF 53R SGIV, fossil ORF in LCDV between positions 59213 and 59611 (AY380826)
099	127708 < 130188	826	94.9			O55768 ORF 184R CIV
100	130333 > 130743	136	15.7	DNA primase	Poxvirus D5-like protein family ATPase (459–765, 86.1%) ^f	AAH75105 <i>Xenopus tropicalis</i>

101	131079 < 131540	153	16.8	DNA double-stranded break repair Rad50-like protein	P loop containing nucleoside triphosphate hydrolase activity SbcC/ATPase (23–153, 14.6%) ^{e,f}	Q91G49 ORF 50L CIV AAAY29120 <i>Phragmotopoma californica</i> Q91G49 ORF 50L CIV
102	131745 > 132350	201	22.6	Exonuclease SbcC subunit C-like protein	P loop containing nucleoside triphosphate hydrolase activity. ^{d,e,f} RecF/RecN/SMc (2–46, 26.5%), SbcC/ATPase (1–677, 74.9%), chromosome segregation (SMC) superfamily (2–46, 3.6%), ^{e,f} ATP-binding protein involved in virulence (2–47, 10.2%) ^e	
103	131663 < 133735	690	78.7		Protein kinase-like (296–391, 55%) ^f	
104	133892 > 135223	443	50.8	Serine/threonine protein kinase		O55720 ORF 98E CIV ABA76540 <i>Pseudomonas fluorescens</i> AAO91711 ORF 129 <i>Choristoneura fumiferana</i> MNPV
105	135233 > 135826	197	22.3	Proline dehydrogenase		
106	136047 < 136244	65	7.3			
107	136277 < 136699	140	16.2	Glyceraldehyde 3-phosphate dehydrogenase-like protein	PutA/delta 1-pyrroline-5 carboxylate dehydrogenase family (6–120, 14%) ^{e,f}	O52485 PutA bifunctional protein
108	137365 < 137883	172	19.9	CTD phosphatase, transcription factor	PS00071 GAPDH active site, one transmembrane helix at the N terminus, one membrane-anchored signal NLI interacting factor-like phosphatase CDT-like phosphatase catalytic domain (27–177, 93.3%), ^e HAD-like domain, ^f TFIIIF-interacting CDT-like phosphatase (25–174, 34.1%)	Q91FF1 ORF 373L CIV Q91FG9 ORF 355R CIV
109	137934 < 138542	202	22.4	ATPase DNA replication, recombination, and repair	P loop containing nucleoside triphosphate hydrolase activity (149–241, 99.3%) ^f one transmembrane helix at the N terminus, Cdc46/MCM2 (148–189, 7.5%) ^e	O55711 ORF 75L CIV
110	138652 < 139824	390	44.8	Putative 1-acylglycerol-3-phosphate acyltransferase	Two transmembrane helices Two transmembrane helices at the N and C termini, PlsC, 1-acyl-sn-glycerol-3-phosphate acyltransferase (20–139, 43.1%), ^e PlsC, phosphate acyltransferase (71–142, 51.5%) ^e	Q6NYV8 Lysocardioliipin acyltransferase
111	140418 > 140690	90	9.3	VLTF2 transcription factor		
112	142112 < 143179	355	39.8			
113	143483 < 144061	192	21.8	Cathepsin B cysteine protease, lysosomal protease	Zinc finger, nucleic acid-binding motif in eEF TFIIIS and RNA polymerases (38–77, 100%) PS00139 eukaryotic thiol protease cysteine active site, PS00639 eukaryotic thiol protease histidine active site, cathepsin B (52–313, 86.9%), ^e peptidase Cl/CIA subfamily (28–315, 93.3%) ^e , Pep C aminopeptidase (212–311, 16%) ^e PS with cut between positions 27/28	Q91FH4 ORF 350L CIV Q91FG3 ORF 361L CIV
114	144146 > 145507	453	50.0	Thioredoxin	One transmembrane helix in the middle	EAL45166 <i>Entamoeba histolytica</i>
115	145449 > 145907	152	16.0	Pyridine nucleotide-disulfide oxidoreductase		
116	145914 > 146405	163	18.3		One transmembrane helix in the middle	AA076649 <i>Bacteroides thetaiotaomicron</i> ZP_00205158 <i>Pseudomonas aeruginosa</i>
117	147038 > 147874	278	32.3	Proline-rich tail		
118	146957 < 147847	296	33.0		PS with cut between positions 22/23	Q9PYT7 ORF 106 <i>Xestia c-nigrum</i> GV AAAX73217 <i>Rickettsia bellii</i> EAL42735 <i>Entamoeba histolytica</i>
119	152174 < 152713	179	19.3	One transmembrane helix in the middle		
120	152712 < 153161	149	16.3			
121	153318 > 153725	135	15.0	One transmembrane helix in the middle		
122	154876 > 155682	268	29.2			
123	156048 < 156824	258	28.6			

^a Putative nonoverlapping ORF selected based on similar codon usage and presence of conserved structural motifs and domains.

^b Positions of amino acid residues and percent alignment with conserved domains in protein families and superfamilies with known functions are shown in parentheses. PS, peptide signal. Iridoviruses: CIV, Chilo iridescent virus; SGIV, Singapore grouper iridescent virus; LCDV, lymphocystis disease virus; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

^c Baculoviruses: MNPV, multiple nucleopolyhedrovirus; SNPV, single nucleopolyhedrovirus; GV, granulovirus.

^d Data from the Pfam database (<http://www.expasy.org/cgi-bin/scanprosite>).

^e Data from the Pfam protein family database (<http://www.sanger.ac.uk/Software/Pfam/>).

^f Data from the Superfamily database (<http://supfam.org/SUPERFAMILY/>).

peptides of 49 to 202 amino acids in length, were found. However, these showed no significant level of homology with other proteins in database searches and lacked conserved domains and motifs (Table 2).

The average protein encoded by these ORFs contained 225 residues. Of these, 70 (57%) were encoded by ORFs present in the forward orientation and 53 (43%) in the reverse. Using a 30-bp window to scan the genome for variations in base pair ratios, we determined that the G+C contents of the 2.9-kbp repeats were lower (~43%) than those for the rest of the genome. Similarly, when the average ORF of 675 bp (3×225 residues) was used as a scanning window to investigate variations in base ratios along the SfAV genome to suppress local nonsignificant differences due to coding capacities, we observed that G+C peaks lower than 46.7% occurred regularly along the sequence (Fig. 2, vertical black arrows on the horizontal axis). All these peaks were located in intergenic regions. Other intergenic regions did not contain lower G+C peaks, indicating that two types of putative intergenic regions were present in SfAV: G+C-rich and G+C-poor regions. Data published previously on transcription of CIV indicate that genes are transcribed temporally as mono- or polycistronic mRNA (31), suggesting that the G+C-poor regions delineate regions in which transcription is arrested at the termination of mRNA synthesis. In agreement with previous ascovirus sequence analyses (97), we observed that all G+C-poor regions contained short inverted repeats that might function as transcription termination signals.

Similarity of SfAV proteins to those of iridoviruses and baculoviruses. Comparison of SfAV genes with those of other large dsDNA viruses that attack insects provided further evidence that this ascovirus shares a common evolutionary lineage with iridoviruses (98, 99). Thirty-five SfAV ORFs (28%) were identified, for which the closest orthologs were those of CIV (Table 1). In addition, 22 (18%) orthologs of baculovirus genes were found in SfAV (Table 1). At least 20 (16%) of the remaining putative proteins shared significant levels of similarity with proteins encoded by eukaryotic genomes (Table 1).

Proteins with conserved domains and motifs and putative functions in SfAV. Compared to other groups of large DNA viruses, ascoviruses have received relatively little study, and thus, the functions of only a few SfAV proteins were known (98). Based on sequence analyses, using the numerical designations herein, these include the major capsid protein (ORF 41), DNA polymerase (ORF 1), thymidine kinase (ORF 40), and ATPase III (ORF 110). Additionally, we recently reported a functional executioner caspase (ORF 73) (16). Based on computer-assisted analyses of the SfAV genome for identification of conserved structural and functional domains and motifs of the various ORFs, as well as their predicted subcellular locations, we were able to assign putative functions to a large number of these proteins (Table 1). Most of the SfAV proteins for which functional assignments could be made were enzymes involved in virus replication, transcription, protein modification, nucleotide metabolism, and virus-host interactions. Additionally, and of potential significance to the unique rescue of apoptotic bodies characteristic of SfAV, we identified several enzymes involved in lipid metabolism not known to occur as a group in other viruses. Below, we describe the

TABLE 2. Properties of small ORFs within the SfAV-1a genome^a

ORF	Nucleotide positions in genome	No. of amino acids	Molecular mass (kDa)
A	3877 < 3359	172	20.0
B	3993 > 4277	94	10.3
C	4836 < 4684	50	5.3
D	8693 > 9187	164	18.7
E	11258 > 11650	130	15.5
F	16620 < 16468	50	6.0
G	17423 > 17572	49	5.6
H	19712 > 19966	84	9.7
I	24274 < 23840	144	15.4
J	30907 < 30689	72	8.4
K	34553 > 34867	104	11.0
L	36129 > 36566	145	16.8
M	41392 < 41183	69	7.9
N	41462 > 41668	68	7.7
O	42152 < 41724	142	16.4
P	42199 > 42555	118	13.8
Q	49244 < 49020	74	8.4
R	49744 < 49367	125	14.0
S	52747 < 52577	56	6.3
T	53306 < 52824	160	18.4
U	66095 > 66337	80	9.0
V	66896 < 66633	87	9.5
W	67601 > 68068	155	18.2
X	68673 > 68837	54	5.9
Y	72677 > 72829	50	5.8
Z	74323 < 73943	126	14.4
AA	76776 > 77246	156	17.4
BB	77176 < 76643	177	19.5
CC	77405 > 77794	129	14.2
DD	77980 < 77480	166	19.2
EE	81334 < 81140	64	7.6
FF	83335 < 83138	65	7.5
GG	84770 < 84609	53	6.0
JJ	90852 > 91013	53	6.0
KK	93734 > 93994	86	10.2
PP	112844 < 112305	179	20.9
QQ	113587 < 112979	202	23.4
RR	114465 > 114737	90	10.8
SS	116073 > 116534	153	17.0
TT	116723 < 116556	55	6.8
UU	117076 < 116744	110	12.8
VV	117122 > 117718	198	22.3
WW	117785 > 118192	135	15.2
XX	118682 < 118203	159	17.5
YY	124942 < 124724	72	8.2
ZZ	125132 < 124950	60	6.8
AAA	130333 > 130743	136	15.7
BBB	130763 > 130936	57	6.3
CCC	137298 < 137087	69	7.7
DDD	146811 < 146620	63	6.8
EEE	149757 > 149948	63	7.2
FFF	150142 < 149954	62	7.2
GGG	150163 > 150324	53	6.0
HHH	150378 > 150593	71	8.2
III	150831 < 150643	62	7.0
JJJ	150918 > 151154	78	8.8
KKK	151155 > 151511	118	13.3
LLL	151868 > 152026	52	6.0
MMM	154384 > 154148	78	9.0
NNN	154603 > 154776	57	6.5
OOO	154768 < 154601	55	6.5

^a These ORFs show no significant homology with other proteins in database searches and contain no conserved domains or motifs. >, forward orientation; <, reverse orientation.

FIG. 2. Variations in G+C content (vertical axis) along the *Spodoptera frugiperda ascovirus 1a* genome (horizontal axis). The graphic representation was calculated using the plot option in Vector NTI (Invitrogen) and a window of 675 nucleotides. G+C-poor intergenic regions are identified by vertical black arrows. Large light gray arrows and a gray box identify the 2.9-kbp repeats, organized as inverted repeats at one locus (spanning the ~80-kbp to 94-kbp positions) and as a 5'- and 3'-truncated repeat at the second locus (spanning the ~147.5-kbp to 149.5-kbp positions). The line located at a G+C content of 46.7% corresponds to the threshold used to differentiate intergenic regions from those putatively transcribed as mono- and polycistronic mRNA. G+C peaks lower than 46.7% along the SfAV-1a genome sequence are indicated by arrows.

apparent functions of these proteins and their likely roles in SfAV replication and pathobiology.

Proteins involved in DNA replication, recombination, and repair. SfAV encodes a δ DNA polymerase (ORF 1) of the B family, which, like the principal replication enzymes in prokaryotes and eukaryotes, contains a nucleotide-polymerizing domain (II-VI-III-I-V) fused to an N-terminal 3' to 5' exonuclease domain (67). No homologue of the proliferating cell nuclear antigen (PCNA)-like clamp, a ring-shaped protein known as a processing factor of DNA polymerase delta, was identified in SfAV. A PCNA-like DNA clamp factor is encoded by other viruses, including vertebrate poxviruses and baculoviruses (56, 81), but as with SfAV, it is not known to occur in entomopoxviruses and iridoviruses. The absence of a PCNA-like clamp protein in SfAV suggests that a processivity factor for DNA replication is provided by the host cell, as is the case for the AcMNPV baculovirus, where the viral PCNA gene is not required for viral replication (81).

SfAV also encodes a homologue (ORF 99, 95 kDa) of the poxvirus D5 family of proteins, which belongs to helicase superfamily III within the AAA+ ATPase class. Helicase superfamily III includes primary replicative helicases encoded by several DNA and RNA viruses (44, 57). The vaccinia virus D5 protein (90 kDa), a nucleic acid-independent nucleoside triphosphatase, is required for viral DNA replication (35). Recently, it has been shown (57) that the poxvirus D5 protein contains a primase domain, which is also present in SfAV ORF 99. The presence and conservation of poxvirus D5 homologues in SfAV and iridoviruses (72) suggest that ORF 99 is essential for viral DNA replication.

Other proteins encoded by SfAV with known or presumed functions in viral DNA replication, recombination, and repair that are typical of large dsDNA viruses include a thymidine kinase (ORF 40), known to be involved in the synthesis of deoxyribonucleotides in cells with suboptimal pools of nucleotides (34, 41); a topoisomerase I (ORF 86), which alters DNA topology by transiently breaking, passing, and rejoining single DNA strands (22, 90); and a DNA ligase (ORF 32) that seals nicked duplex DNA substrates (95). Other SfAV enzymes that likely play roles in DNA metabolism include an ATPase (ORF 110), ssDNA and dsDNA nucleases (ORF 37 and ORF 75), a helicase (ORF 95), serine/threonine kinases (ORF 64, ORF 82, and ORF 104), and a tyrosine kinase (ORF 90) (68, 74) as

well as members of the Rec/Sbc/Rad superfamilies. These include RecD exonuclease V (ORF 95), SbcC-like and SbcD-like exonucleases (ORF 59 and ORF 103, respectively), and a Rad50-like nuclease (ORF 101) (2, 26, 35, 80, 94, 104).

SfAV also encodes a putative FEN-1/FLAP-like nuclease (ORF 66). FEN-1/FLAP nucleases share structural and functional similarities with eubacterial DNA polymerases, Rad2, and the *Xeroderma pigmentosum* G (XPG N and I regions) repair endonuclease and also possess RNase H activity (102). Homologues of ORF 66 are also present in iridovirus, poxvirus, *Emiliania huxleyi* virus, and mimivirus, all of which are among the large nuclear and cytoplasmic DNA viruses, the so-called NCLDV family (56). A FEN-1/FLAP homologue is known to function in RNA primer removal during T4 phage DNA replication, and in poxvirus, a homologue (G5R) apparently functions in the early stage of viral morphogenesis (30, 56). Interestingly, it has been shown that the homologous enzyme in herpesviruses inhibits cellular gene expression in infected cells, destabilizing preexisting host mRNAs and thereby ensuring rapid turnover of viral mRNA. Thus, ORF 66 could have several functions in SfAV virogenesis.

Genes involved in transcription and RNA metabolism. SfAV gene products that apparently participate in mRNA biogenesis included the DNA-dependent RNA polymerase subunit C (ORF 8) and RNA polymerase II subunits 1 and 2 (ORF 67 and ORF 52, respectively). In addition, three transcription factor-like proteins, TFIIF (ORF 109), VLTF2 (virus-like transcription factor 2, ORF 113), and Yabby-like TF (ORF 91), were encoded by SfAV. The Yabby-like proteins in plants constitute a group of putative transcription factors with a Cys-containing zinc finger. These proteins also contain a DNA-binding domain with high similarity to the HMG (high-mobility-group) family of transcription factors (73). As Yabby proteins participate in abaxial-adaxial identity determination in lateral organs in *Arabidopsis thaliana*, and HMG proteins are known to modulate gene expression by interacting with nucleosomes, transcription factors, nucleosome-remodelling machines, and histone H1 (14), SfAV ORF 91 could have similar functions. Although a homologue of ORF 91 is present in CIV (T03180), the function of this protein is unknown. In our database searches, no homologues were identified in other virus families.

SfAV ORF 66, which has a Fen-1/FLAP-like nuclease do-

main (noted above), also contains a motif within the XPG N-I regions that corresponds to sequences found in various virion host shutoff (VHS) proteins encoded by herpesviruses, varicella-zoster virus, and pseudorabies virus. VHS proteins inhibit cellular gene expression in infected cells and destabilize pre-existing host mRNAs, thereby ensuring rapid turnover of virus-specific mRNAs (69, 93).

In addition, SfAV contains two genes (ORF 22 and ORF 23) that encode proteins with similarities to prokaryotic RNase III. ORF 22 contains a well-conserved RNase III catalytic domain that is required for cleavage of dsRNA templates. In prokaryotes and eukaryotes, RNase III participates in a variety of functions, including the processing of cellular and virus-encoded precursor RNAs into mRNAs, rRNAs, and tRNAs, and also is involved in the degradation of specific mRNAs (33, 79). The RNase III catalytic domain is also present in Dicer, also a member of the RNase III protein superfamily, which cleaves precursor dsRNA into small temporal RNAs and short interfering RNA (siRNA) of ~22 nucleotides (5, 79). siRNAs participate in gene-specific inactivation, whereas small temporal RNAs function in the control of developmental processes (79).

Database searches indicated that SfAV RNase III homologues are not encoded by dsDNA viruses other than ascoviruses and the iridescent virus *Paramecium bursaria* chlorella virus 1 (family *Phycodnaviridae*). Zhang et al. (111) have shown that the *Paramecium bursaria* chlorella virus 1 RNase III is an active enzyme, but as this protein lacks additional N-terminal domains (ATPase/helicase, DUF283, and PAZ) present in Dicer proteins (79, 110), it is unlikely that the viral RNase III functions in gene-specific silencing during virogenesis. Interestingly, however, it has been shown that an RNase III encoded by the sweet potato chlorotic stunt virus, an RNA virus (family *Closteroviridae*), enhances suppression of host-induced RNA silencing by a sweet potato chlorotic stunt virus-encoded p22 protein (66). Thus, it is possible that the RNase III encoded by these dsDNA viruses could function in a similar way to evade an antiviral response elicited by host-directed siRNA.

Cellular homologues involved in protein modification, processing, and apoptosis. (i) **Protein kinases and oxidoreductases.** SfAV codes for several enzymes that apparently modify proteins by phosphorylation/dephosphorylation and oxidation/reduction processes. SfAV protein kinases potentially involved in both upregulating and downregulating diverse cellular regulatory processes and pathways, including transcription, translation, and cell division, include a tyrosine protein kinase (ORF 90), two serine/threonine kinases (ORF 64 and ORF 104), and a thymidine kinase (ORF 40).

ORFs related to the vaccinia virus thiol oxidoreductase (E10R) and thioredoxin (G4L) are also present in iridoviruses, poxviruses, and other large nuclear and cytoplasmic viruses (45, 72, 87, 88). Thiol-oxidase-like proteins (ORF 61 and ORF 116) are also encoded by SfAV. These enzymes are members of the Evr1/Alr (Evr1, essential for respiration and vegetative growth; Alr, augmentor or liver regeneration) family of flavin adenine dinucleotide-containing sulfhydryl oxidases that use oxygen as an electron acceptor. Evr1/Alr proteins contain a conserved domain of about 100 amino acid residues with an invariant C-X-X-C active-site motif that is implicated in redox function (86, 87, 88). Representatives of the Evr1/Alr proteins

include the *Saccharomyces cerevisiae* Evr1 protein, required for mitochondrial development, and mammalian hematopoietin (ALR). The G4L has thiol transferase activity and is required for formation of disulfide bonds (108). Recently, Senkevich et al. (87, 88) have shown that E10R and G4L function in the formation of disulfide bonds between conserved cysteine residues of viral structural membrane-bound proteins and are required for virion morphogenesis. Thus, the SfAV ORF 61 and ORF 116 proteins may play a similar role in proteins bound to the virion membrane.

(ii) **IAPs.** SfAV encodes four inhibitor of apoptosis protein (IAP)-like proteins (ORF 15, ORF 16, ORF 25, and ORF 74). The SfAV-1a IAP-like homologues ORF 16, ORF 25, and ORF 74 are most closely related to cellular proteins from *Danio rerio* (zebrafish), *Xenopus laevis* (African clawed frog), and rodent, respectively, whereas ORF 15 is most closely related to a baculovirus IAP. Nevertheless, based on phylogenetic studies, baculovirus IAPs are thought to have been derived from lepidopteran hosts (54). The presence of at least four IAP-like proteins in the SfAV genome is not unusual. In baculovirus genomes, from one to four IAP-like proteins have been reported (55), and in the invertebrate CIV genome, two copies are present (58). IAPs are metalloproteins that contain one or more zinc-binding motifs referred to as baculovirus IAP repeats and a RING (really interesting new gene) domain (105). First described in baculoviruses (24, 27), cellular homologues of IAPs have been reported in organisms ranging from yeasts to humans and, most recently, plants (51). Many IAPs are known to function by directly inhibiting caspases, thereby halting the execution phase of programmed cell death (105) and maximizing efficiency of viral replication (24). Thus, SfAV IAPs could function in the transient regulation of the apoptotic response. One possibility is that shortly after infection, one or more of the SfAV IAPs act in a cascade in which apoptosis is initially inhibited to enable the ascovirus to gain access to the nucleus and establish infection, after which the caspase gene is expressed to trigger cell death and initiate the development of apoptotic bodies. Other possible roles for SfAV IAPs include posttranslational modification and degradation of proteins via ubiquitination (see below).

(iii) **E3 ubiquitin ligases and IAPs.** The ubiquitin-proteasome system is a well-conserved system in metazoans that is used for posttranslational modification and degradation of proteins. Three essential components are required in a cascade that targets proteins for ubiquitination: E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin ligase (77). E1 transfers ubiquitin, a protein of 76 amino acid residues, to E2 ubiquitin, which, together with E3, ligates ubiquitin to targets doomed for degradation by proteasomes. E3 ubiquitin ligase contains a zinc-binding motif called a RING domain that is essential for ubiquitination (6). SfAV ORFs 16, 81, and 97 encode proteins with conserved RING domains that are characteristic of the E3 ubiquitin ligase. Members of the nucleo-cytoplasmic large DNA viruses are also known to encode ubiquitin homologues (56).

The Op-IAP3 baculovirus IAP (inhibitor of apoptosis protein; see above), which also contains a RING (C3H4) domain (105), functions as an E3 ubiquitin protein ligase, as shown recently (75). As noted above, the SfAV genome encodes four IAPs (ORF 15, ORF 16, ORF 25, and ORF 74), which there-

fore could potentially function in the ubiquitin pathway. Interestingly, a breast- and ovarian-specific tumor suppressor-like protein (BRCA-1) is also encoded by SfAV ORF 97. BRCA-1, together with another protein (BARD1), has been shown to function as an E3 ubiquitin ligase (96). Ubiquitin posttranslational protein modification is involved in numerous cellular processes, including signal transduction, the cell cycle, and programmed cell death, in the last of which the E3 ubiquitin ligase targets include the proapoptotic proteins, including Hid, Reaper, Grim, and caspases (75). Alternatively, more-recent data suggest that an E3 ubiquitin ligase encoded by the white spot syndrome virus functions as an inhibitor of apoptosis to ensure efficient virus propagation (48). Thus, it is possible that the SfAV E3-ubiquitin ligases and the IAP-like and BRCA-1-like proteins all play similar roles during SfAV replication.

Proapoptotic proteins. (i) Executioner caspase. The cytopathology induced by most ascoviruses is novel among viruses in that virogenesis involves a modified form of cell death in which apoptotic bodies, as they develop, are converted into virion-containing vesicles (36, 39). This suggested that SfAV encoded proteins unique to this virus type that induce apoptosis and rescue the developing apoptotic bodies. As noted above, we demonstrated recently that SfAV encodes a functional executioner caspase (ORF 73) that apparently plays a direct role in initiating apoptosis (16). As executioner caspases also are known to be involved in the maturation of viral proteins (13), the SfAV caspase may also be involved in virion maturation.

(ii) Cathepsin B (SfAV ORF 114). Although caspases are the key enzymes that regulate programmed cell death, over the past few years it has been shown that cathepsins, particularly cathepsins B, D, and L, also play crucial roles in this process (23). Cathepsins are cysteine proteases that belong to the C1 family of enzymes, the so-called papain family. These enzymes are synthesized as zymogens and are glycosylated posttranslationally, after which they are compartmentalized in lysosomes, a process mediated by cellular mannose-6-phosphate receptors. Activated cathepsins released from lysosomes through various stimuli, including sphingomyelin diphosphodiesterase (SMase) activity (see below), direct many cellular pathways, such as protein degradation, proenzyme activation, antigen processing, metabolism, and apoptosis. With regard to proapoptotic mechanisms, it is interesting to note that SfAV encodes a protein (ORF 114) that contains a well-conserved peptidase domain found in cathepsin B. Recent evidence suggests that cathepsin B could regulate apoptosis by attacking the mitochondria directly causing the release of cytochrome *c* or by activating proapoptotic proteins of the Bcl2 superfamily, such as Bid and Bax (23), that activate initiator caspases, which in turn activate executioner caspases and possibly the SfAV caspase (ORF 73). Cathepsin homologues are also known in baculoviruses and the CIV (58, 92). The function of the cathepsin B homologue in CIV is unknown. However, the baculovirus cathepsin L homologue is thought to be linked to degradation and liquefaction of host tissues during virus pathogenesis (47, 92).

Enzymes involved in lipid metabolism. A unique feature of SfAV and other ascoviruses, such as TnAV-2a and HvAV-3a, is that after nuclear lysis and host DNA degradation, cell cleavage similar to apoptosis occurs. However, instead of forming typical apoptotic bodies, the cell cleaves into numerous

virion-containing vesicles, each of which occludes numerous virions in various stages of morphogenesis along with a virogenic stroma, apparently still active. A characteristic of this process is that de novo membrane synthesis occurs as these virion-containing vesicles form. Most host cell DNA is degraded by the time the viral vesicles form, suggesting that the elaboration of membranes that delimit these vesicles may be a product of lipid-metabolizing enzymes encoded by SfAV. Consequently, our search of the SfAV genome revealed genes encoding the following enzymes, known to play a role in lipid metabolism. Whereas some of these occur individually in other viral genomes, they do not occur together in any other viral genome.

(i) SfAV ORF 87, encoding fatty acid elongase (ELO). The SfAV ORF 87 protein belongs to the GSN1/SUR4 enzyme family (11), which catalyzes the biosynthesis of long-chain (26 carbons) fatty acid precursors for ceramide and sphingoid lipogenesis. In yeast, allelic variants of ELO2 and ELO3 (VBM1 and VBM2, respectively), also involved in long-chain fatty acid elongation and sphingoid synthesis, appear to play an important role in protein sorting and trafficking of secretory vesicles (59). A gene homologue (AJ58127) is present in the fowlpox virus.

(ii) SfAV ORF 59, encoding sphingomyelin phosphodiesterase. SfAV ORF 59, which also contains an SbcD DNA repair exonuclease domain (see above), has a well-conserved overlapping calcineurin-like phosphoesterase domain found in a wide variety of phosphoesterases, including protein phosphoserine phosphatases, nucleotidases, 2'-3' cyclic AMP phosphodiesterase, and SMases (4). SMases cleave sphingomyelin to release phosphocholine and ceramide. A gene homologue (Q91FS8) is present in CIV.

(iii) SfAV ORF 112, encoding phosphate acyltransferase. A conserved domain in SfAV ORF 112 suggests that this protein belongs to the phosphate acyltransferase (PlsC) family of proteins. Members of this family, which include 1-acylglycerol-3-phosphate acyltransferase, function in de novo biosynthesis of cell membrane phospholipids and have glycerophosphate, 1-acylglycerolphosphate, or 2-acylglycerolphosphoethanolamine acyltransferase activities (1, 29). No viral homologues of this enzyme were identified in database searches.

(iv) SfAV ORF 93, encoding a patatin-like phospholipase/alpha/beta hydrolase. The SfAV ORF 93 protein contains a FabD/lysophospholipase-like domain related to the patatin-like phospholipases (53, 83). A homologue (Q91F63) is present in CIV. The patatin domain overlaps with a highly conserved domain found in members of the alpha/beta hydrolase fold superfamily of enzymes. These enzymes have diverse catalytic functions, including lipolytic, thioesterase, and peptidase activities. Patatin, a storage protein in potato tubers, also has an intrinsic lipid acylhydrolase activity that catalyzes the cleavage of fatty acids from membrane lipids. The structural characteristics of the active site in patatin, which contains a Ser-Asp dyad, are similar to those of cytosolic phospholipase A₂ (PLA₂) (53). The PLA₂ family of enzymes plays a central role in several cellular processes, such as phospholipid digestion and metabolism, host defense, and signal transduction (52, 112), and is also implicated in the induction of apoptosis via ceramide biogenesis (7, 60) and nuclear shrinkage in caspase-independent cell death (89). Interestingly, parvovirus capsid

proteins have an intrinsic PLA₂-like activity required for endocytic entry of the virus (21, 101), a possible function of ORF 93. In addition, patatin was shown to inhibit growth of the corn root worm (*Diabrotica* sp.) (100). Ascovirus-infected larvae exhibit decreased feeding and growth, and though unlikely, this patatin-like protein may be partly responsible for this characteristic.

(v) **SfAV ORF 13, encoding carboxylesterase/JHE.** The SfAV ORF 13 protein contains a well-conserved carboxylesterase domain found in the alpha/beta hydrolase lipase family and is also related to the juvenile hormone esterase (JHE) of *Apis mellifera*. Carboxylesterases are lipases that catalyze the hydrolysis of a wide variety of ester- and amide-containing molecules, including short- and long-chain acylglycerols, long-chain acylcarnitine, and long-chain acyl-coenzyme A esters (85). JHE degrades the juvenile hormone, which plays diverse roles in the insect life cycle, including development, morphogenesis, reproduction, diapause, and metabolism (43, 62). No other viral homologues were identified in database searches.

Lipid metabolism and SfAV-induced apoptosis. The presence of a large number of genes coding for enzymes involved in lipid metabolism is unusual for known prokaryotic and eukaryotic viruses. Collectively, these enzymes could play important roles in the reorganization of existing membranes and de novo membrane synthesis during SfAV maturation (38) and in initiating and mediating the apoptotic response through sphingoid signaling. A number of studies have linked oxidative stress induced by reactive oxygen species with ceramide generation during the apoptotic program (4, 91), and it is well established that ceramide is an endogenous regulator of apoptosis (20, 65). In this regard, it is interesting to note that lysosomal permeabilization and cathepsin B release (see above) can be initiated by a number of extralysosomal and intralysosomal stimuli that include oxidative stress induced by reactive oxygen species and, in particular, by sphingoid derivatives, especially ceramides, produced by sphingomyelinase and ceramidase enzymatic activities (4, 23). Recently, it has also been shown that apoptosis induced by endoplasmic reticulum stress in insulin-secreting cells is amplified by overexpression of phospholipase A₂ (iPLA₂B) (82). Based on these observations, it is tempting to speculate that the putative SfAV cathepsin B, SMase, patatin-like PLA₂, and SfAV caspase function in concert to provide a pathway that leads to the rapid onset of the apoptotic response observed in SfAV-infected cells and larvae (16, 36).

The SfAV viral lipases, particularly the patatin-like phospholipase and coesterase/JHE alpha/beta hydrolases, could also function in viral membrane biogenesis and egress of virus from the cell. In this regard, Baek et al. (9) have shown that the major envelope protein (p37) encoded by the vaccinia virus is a lipase that participates in the de novo synthesis of the double membranes of this virus and is also involved in viral entry and exit from infected cells.

Enzymes with other functions. (i) SfAV ORF 107, encoding PutA. The SfAV ORF 107 protein contains a domain conserved in the PutA family of NAD-dependent aldehyde dehydrogenases. Members of the PutA family in prokaryotes and eukaryotes have two active enzymes, proline oxidase and delta 1-pyrroline-5-carboxylate dehydrogenase. In eukaryotes, proline oxidase is located on the inner mitochondrial membrane, is involved in the proline/pyrroline-5-carboxylate cycle, and

catalyzes the formation of reactive oxygen species. Oxidative stress induced by reactive oxygen species and the accumulation of pyrroline-5-carboxylate have been shown to induce and/or contribute to the apoptotic response (32, 70, 71).

(ii) **SfAV ORF 14, encoding matrixin.** The SfAV ORF 14 protein contains a zinc-binding motif (HEXGHXXGXXH) in the catalytic domain conserved in the matrixin zinc-dependent family of metalloproteases. These proteins, which include collagenases and gelatinases that cleave denatured collagens, are peptidases that participate in the degradation of the extracellular matrix (106). A possible role for this protein could be the degradation of the extracellular matrix surrounding adipocytes, a cellular target of SfAV (39, 46).

(iii) **Baculovirus repeated open reading frame genes (*bro*).** The *bro* genes, first identified in baculoviruses, constitute a multigene family typically with many copies per genome and are also known to occur among other large insect dsDNA viruses and bacteriophages (15). These sequences are absent in vertebrate genomes and vertebrate viruses. Although a few baculoviruses lack *bro* homologues, others are known to contain from 1 to 16 copies that are either active or inactive genes (15). SfAV contains seven *bro*-like gene homologues that encode from 97 to 363 amino acid residues (ORFs 30, 31, 69, 70, 72, 79, and 80). We observed that three of these appeared to be alleles, suggesting that variations occurred by mutation and recombination in this ascovirus. Little is known about the function(s) of BRO proteins in viral biology, but at least one baculovirus BRO protein has been shown to bind nucleic acid, associate with nucleosomes, and interact with host cell laminin (63, 109). In another study (15), inactivation of a single-copy *bro* gene in a baculovirus showed that it was not essential for virus replication but may enhance replication during the late phase of the viral life cycle.

(iv) **SfAV ORF 46, encoding a protein with multifunctional domains.** The putative protein encoded by ORF 46 contains several well-conserved overlapping domains (Smc, Hec1, SbcC, filament, and Reo_Sigma1). The Smc family consists of chromosome segregation ATPases that function during the cell division cycle (78). The Hec1 proteins are known to interact with Smc proteins (64). The SbcC ATPases are involved in DNA replication, recombination, and repair (2, 26, 35, 80, 94, 104). The Reo_Sigma1 family is composed of glycoproteins that share homology with the reoviral sigma1 hemagglutinin cell attachment protein, a minor capsid protein that determines serotype-specific humoral response and is also involved in modulating the apoptotic response in reovirus-infected cells (10, 42).

SfAV ORF 46 also contains a stretch of amino acid residues with high homology to the cytoskeletal intermediate-filament protein signature domain (50). The critical role of cytoskeletal proteins, particularly the involvement of intermediate filaments in virion maturation and release from infected cells, is well established (28, 49, 76). The assembly of large DNA viruses, such as poxviruses, iridoviruses, and the closely related African swine fever virus, occurs in the cytoplasm in viral factories and aggresomes that recruit mitochondria, causing the rearrangement of intermediate filaments and the collapse of vimentin into cage-like structures (49). Cordo and Candurra (25) have also shown that intermediate filament integrity is essential for replication of the Junin virus. As apoptosis, which

includes cytoskeletal disintegration and reorganization, proceeds rapidly during SfAV infection, the cytoskeletal network that could be essential for SfAV maturation may not be available. Thus, the putative intermediate filament protein encoded by SfAV ORF 46 could play an essential role in forming scaffolding networks for viral assembly and maturation. Viral gene homologues of SfAV ORF 46 occur in granuloviruses (family *Baculoviridae*) of *Cydia pomonella* (U82510) and *Phthorimaea operculella* (AF499596), viruses that replicate in the cytoplasm of infected cells.

Comparison of SfAV-1a and TnAV-2c genomes. The genomic sequence of *Trichoplusia ni* ascovirus 2c (TnAV-2c) has recently been published (107). The genome of TnAV-2c is circular, consists of 174,059 bp, with a G+C content of 35.4%, and putatively encodes 165 proteins. This makes the TnAV-2c genome approximately 11% larger than that of SfAV-1a. Based on the putative annotated proteins coded for by TnAV-2c and SfAV-1a, homologues shared by both viruses include those involved in virion structure (major capsid protein), nucleic acid metabolism (delta DNA polymerase, RNA polymerase, ATPases, RNase III, and nucleases), and inhibitor of apoptosis (IAPs) (Table 3). With respect to the unique cytopathology of ascoviruses, TnAV-2c also contains ORFs that putatively encode enzymes involved in promoting apoptosis and viral vesicle formation, as described above for SfAV. These proteins include a caspase, cathepsin B, and enzymes involved in lipid metabolism (patatin-like phospholipase, PlsC phosphate acyltransferase, fatty acid elongase, and lipase) (Table 3). Apparent genes that occur in SfAV but not in TnAV-2c include those that putatively encode SbcC and RecD V exonucleases, a UrvD/Rep helicase, serine/threonine kinases, an executioner caspase, and a PutA/proline dehydrogenase.

In summary, the genome of SfAV encodes proteins that would be expected based on what is known about the biochemical characteristics of the virions and the replication of this virus initially in the nucleus and then in a mixture of nucleoplasm and cytoplasm. These proteins include various DNA and RNA polymerases, helicases, kinases, and structural proteins, such as the capsid protein. In addition, various other proteins consistent with the unique cytopathology of ascoviruses that are either rare or absent from other known viruses were present. These include an executioner caspase, cathepsin B, and a complex of enzymes that modify lipids. The presence of genes for these enzymes, which are absent or rare in other viruses and do not occur as a group, suggests that they are directly involved in rescuing apoptotic bodies and converting them into virion-containing vesicles. Although the interactions of this diverse set of enzymes and structural proteins are obviously complex, the availability of the SfAV genomic sequence provides a foundation upon which the role of these various proteins in viral reproduction and pathogenesis can be studied. Aside from contributions to virology, such studies should contribute to our understanding of the regulation and manipulation of apoptosis as well as cell biology and lateral gene transfer during ascovirus evolution. With respect to the last, the relationship we detected between SfAV-1a and *Chilo* IV, an iridovirus that infects lepidopterans, suggests that many genes coding for proteins involved in ascovirus vesicle formation or their homologues may have been acquired from a lepidopteran

TABLE 3. Putative ORFs common to SfAV-1a and TnAV-2c^a

ORF group	ORF(s) in indicated gene	
	SfAV-1a	TnAV-2c
Nucleotide metabolism		
Delta DNA polymerase	1	1
DNA-dependent RNA polymerase	8	118
SNF2 DEAD-like helicase	9	161
RNase III	22	8
DNA ligase	32	85
GIY-YIG endonuclease	37	87
Thymidine kinase	40	146, 154
DNA-dependent RNA polymerase II, subunit 11	52	110, 42
FEN-1/FLAP, XPG RAD2-like endonuclease	66	112
SI/PI nuclease	75	135
ATPase III	110	95
Transcription factor TFIIS	113	99
Lipid metabolism		
Esterase/lipase	13	132
Fatty acid elongase	87	46
Patatin-like phospholipase	93	67
PlsC, phosphate acyltransferase	112	98
Apoptosis		
Inhibitor of apoptosis (IAP)	15, 16, 25, 74	104
Cathepsin B	114	102
Others		
Metalloproteinase	14	158
Baculovirus repeated open reading frames (BRO)	30, 31 69, 70 72, 79 80	27, ORF 137 165
Major capsid protein	41	153

^a Comparisons based on description of TnAV-2c genome by Wang et al. (107).

host, as CIV does not form such vesicles. An alternative hypothesis is that these genes originated from the genome of a parasitic wasp, such as the ichneumonid *D. pulchellus*, that transmits DpAV-4a, the genome of which has been detected in nuclei of its wasp vector without causing any apparent pathology (17). These hypotheses are clearly speculative and will be answered only upon more experimental research with ascovirus and the determination of full genomic sequences for several endoparasitic wasp and lepidopteran species. Nevertheless, as no other virus undergoes the apoptotic-like process characteristic of ascoviruses, it is probable that the key genes involved in this process originated by lateral gene transfer from either a lepidopteran or a hymenopteran host.

ACKNOWLEDGMENTS

This research was funded in part by grants to B. A. Federici from the United States National Science Foundation (INT-9726818) and to Y. Bigot from the CNRS, the Groupement de Recherche CNRS 2157, the Institut Fédératif de Recherche 136, the Ministère de l'Éducation Nationale, de la Recherche et de la Technologie, and the University François Rabelais of Tours.

We thank J. J. Johnson for his assistance throughout our investigations.

REFERENCES

1. Agarwal, A. K., R. A. Barnes, and A. Garg. 2006. Functional characterization of human 1-acylglycerol-3-phosphate acyltransferase isoform 8: cloning, tissue distribution, gene structure, and enzyme activity. *Arch. Biochem. Biophys.* 449:64–76.
2. Ahmad, S. I., S. H. Kirk, and A. Eisenstark. 1998. Thymine metabolism and

- thymineless death in prokaryotes and eukaryotes. *Annu. Rev. Microbiol.* **52**:591–625.
3. Alfonso, C. L., E. R. Tulman, Z. Lu, E. Oma, G. F. Kutish, and B. L. Rock. 1999. The genome of *Melanoplus* entomopoxvirus. *J. Virol.* **73**:533–552.
 4. Andrieu-Abadie, N., and T. Lavade. 2002. Spingomyelin hydrolysis during apoptosis. *Biochim. Biophys. Acta* **1585**:126–134.
 5. Aravind, L., and E. V. Koonin. 2001. A natural classification of ribonucleases. *Methods Enzymol.* **341**:3–28.
 6. Ardley, H. C., and P. A. Robinson. 2005. E3 ubiquitin ligase. *Essays Biochem.* **41**:15–30.
 7. Atsumi, G., M. Murakami, K. Kojima, A. Hadano, M. Tajima, and I. Kudo. 2000. Distinct roles of two intracellular phospholipase A₂s in fatty acid release in the cell death pathway. *J. Biol. Chem.* **274**:18248–18258.
 8. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1994. Current protocols in molecular biology, vol. 1 and 2. John Wiley, New York, N.Y.
 9. Baek, S. H., J. Y. Kwak, S. H. Lee, T. Lee, S. H. Ryu, D. J. Uhlinger, and J. D. Lambeth. 1997. Lipase activities of p37, the major envelope protein of vaccinia virus. *J. Biol. Chem.* **272**:32042–32049.
 10. Barton, E. S., J. D. Chappell, J. L. Connolly, J. C. Forrest, and T. S. Dermody. 2001. Reovirus receptors and apoptosis. *Virology* **290**:173–180.
 11. Baudry, K., E. Swain, A. Rahier, M. Germann, A. Batta, S. Rondet, S. Mandala, K. Henry, G. S. Tint, T. Edlind, M. Kurtz, and J. T. Nickels, Jr. 2001. The effects of the *erg26-1* mutation on the regulation of lipid metabolism in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **276**:12702–12711.
 12. Bawden, A. L., K. J. Glassberg, J. Diggans, R. Shaw, W. Farmerie, and R. W. Moyer. 2000. Complete genomic sequence of the *Amsacta moorei* entomopoxvirus: analysis and comparison with other poxviruses. *Virology* **274**:120–139.
 13. Best, S. M., and M. E. Bloom. 2004. Caspase activation during virus infection: more than just a kiss of death? *Virology* **320**:191–194.
 14. Bianchi, M. E., and A. Agresti. 2005. HMG proteins: dynamic players in gene regulation and differentiation. *Curr. Opin. Genet. Dev.* **15**:496–506.
 15. Bideshi, D. K., S. Renault, K. Stasiak, B. A. Federici, and Y. Bigot. 2003. Phylogenetic analysis and possible function of *bro*-like genes, a multigene family widespread among large double-stranded DNA viruses of invertebrates and bacteria. *J. Gen. Virol.* **84**:2531–2544.
 16. Bideshi, D. K., Y. Tan, Y. Bigot, and B. A. Federici. 2005. A viral caspase contributes to modified apoptosis for virus transmission. *Genes Dev.* **19**:1416–1421.
 17. Bigot, Y., A. Rabouille, G. Doury, P. Y. Sizaret, F. Delbost, M. H. Hamelin, and G. Periquet. 1997. Biological and molecular features of the relationships between *Diadromus pulchellus* ascovirus, a parasitoid hymenopteran wasp (*Diadromus pulchellus*) and its lepidopteran host, *Acrolepiopsis assectella*. *J. Gen. Virol.* **78**:1149–1163.
 18. Bigot, Y., A. Rabouille, P. Y. Sizaret, M. H. Hamelin, and G. Periquet. 1997. Particle and genomic characterization of a new member of the *Ascoviridae*, *Diadromus pulchellus* ascovirus. *J. Gen. Virol.* **78**:1139–1147.
 19. Bigot, Y., K. Stasiak, F. Rouleux-Bonnin, and B. A. Federici. 2000. Characterization of repetitive DNA regions and methylated DNA in ascovirus genomes. *J. Gen. Virol.* **81**:3073–3082.
 20. Birbes, H., S. El Bawab, Y. A. Hannun, and L. M. Obied. 2001. Selective hydrolysis of a mitochondrial pool of spingomyelin induces apoptosis. *FASEB J.* **14**:2669–2679.
 21. Canaan, S., Z. Zadori, F. Ghomashchi, J. Bollinger, M. Sadilek, M. E. Moreau, P. Tijssen, and M. H. Gelb. 2004. Interfacial enzymology of parvovirus phospholipase A₂. *J. Biol. Chem.* **279**:14502–14508.
 22. Champoux, J. J. 1990. Mechanistic aspects of type-1 topoisomerases, p. 217–242. In N. R. Cozzarelli and J. C. Wang (ed.), DNA topology and its biological effects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 23. Chwieralski, C. E., T. Welte, and F. Buhling. 2006. Cathepsin-regulated apoptosis. *Apoptosis* **11**:143–149.
 24. Clem, R. J. 2001. Baculovirus and apoptosis: the good, the bad, and the ugly. *Cell Death Differ.* **8**:137–143.
 25. Cordo, S. M., and N. A. Candurra. 2003. Intermediate filament integrity is required for Junin virus replication. *Virus Res.* **97**:47–55.
 26. Cromie, G. A., J. C. Connelly, and D. R. Leach. 2001. Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. *Mol. Cell* **8**:1163–1174.
 27. Crooke, 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.
 28. Cudmore, S., I. Reckmann, and M. Way. 1997. Viral manipulations of the actin cytoskeleton. *Trends Microbiol.* **5**:142–148.
 29. Cullinane, M., C. Bayse, J. P. Morrissey, and F. O'Gara. 2005. Identification of two lysophosphatidic acid acyltransferase genes with overlapping functions in *Pseudomonas fluorescens*. *Microbiology* **151**:3071–3080.
 30. da Fonseca, F. G., A. S. Weisberg, M. F. Caeiro, and B. Moss. 2004. Vaccinia virus mutants with alanine substitutions in the conserved G5R gene fail to initiate morphogenesis at the nonpermissive temperature. *J. Virol.* **78**:10238–10248.
 31. D'Costa, S. M., H. J. Yao, and S. L. Bilimoria. 2004. Transcriptional mapping of Chilo iridescent virus infections. *Arch. Virol.* **149**:723–742.
 32. Deuschle, K., D. Funck, G. Forlani, H. Stransky, A. Biehl, S. Leister, E. Van der Graaff, R. Kunze, and W. B. Frommer. 2004. The role of delta 1-pyrroline-5-carboxylate dehydrogenase in proline degradation. *Plant Cell* **16**:3413–3425.
 33. Drider, D., and C. Condon. 2004. The continuing story of endoribonuclease III. *J. Mol. Microbiol. Biotechnol.* **8**:195–200.
 34. Efsthathiou, S., D. Kemp, G. Darby, and A. C. Minson. 1989. The role of herpes simplex virus type 1 thymidine kinase in pathogenesis. *J. Gen. Virol.* **70**:869–879.
 35. Evans, E., N. Klemperer, R. Ghosh, and P. Traktman. 1995. The vaccinia virus D5 protein, which is required for DNA replication, is a nucleic acid-independent nucleoside triphosphate. *J. Virol.* **69**:5353–5361.
 36. Federici, B. A. 1983. Enveloped double-stranded DNA insect virus with novel structure and cytopathology. *Proc. Natl. Acad. Sci. USA* **80**:7664–7668.
 37. Federici, B. A., Y. Bigot, R. R. Granados, J. J. Hamm, L. K. Miller, I. Newton, K. Stasiak, and J. M. Vlak. 2005. Family *Ascoviridae*, p. 247–250. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball (ed.), Virus taxonomy. Eighth report of the International Committee on Taxonomy of Viruses. Elsevier, Amsterdam, The Netherlands.
 38. Federici, B. A., Y. Bigot, R. R. Granados, J. J. Hamm, L. K. Miller, and J. M. Vlak. 2000. Family *Ascoviridae*, p. 261–265. In M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop, G. B. Carstens, M. K. Estes, S. M. Lemon, J. Maniloff, M. A. Mayo, D. J. McGeoch, C. R. Pringle, and R. B. Wickner (ed.), Taxonomy of viruses. Seventh report of the International Committee on Virus Taxonomy. Academic Press, London, United Kingdom.
 39. Federici, B. A., and R. Govindarajan. 1990. Comparative histopathology of three ascovirus isolates in larval noctuids. *J. Invertebr. Pathol.* **56**:300–311.
 40. Federici, B. A., J. M. Vlak, and J. J. Hamm. 1990. Comparative study of virion structure, protein composition and genomic DNA of three ascovirus isolates. *J. Gen. Virol.* **71**:1661–1668.
 41. Field, H. J., and P. Wildy. 1978. The pathogenicity of thymidine kinase-deficient mutants of herpes simplex virus in mice. *J. Hyg.* **81**:267–277.
 42. Forrest, J. C., and T. S. Dermody. 2003. Reovirus receptors and pathogenesis. *J. Virol.* **77**:9109–9115.
 43. Gilbert, L. I., N. A. Granger, and R. M. Roe. 2000. The juvenile hormones, historical facts and speculations on future research directions. *Insect Biochem. Mol. Biol.* **20**:617–644.
 44. Gorbalenya, A. E., E. V. Koonin, and Y. I. Wolf. 1990. A new superfamily of putative NTP-binding domains encoded by genomes of small DNA and RNA viruses. *FEBS Lett.* **262**:145–148.
 45. Gvakharia, B. O., E. K. Koonin, and C. K. Mathews. 1996. Vaccinia virus G41 gene encodes a glutaredoxin. *Virology* **226**:408–411.
 46. Hamm, J. J., E. L. Styer, and B. A. Federici. 1998. Comparison of field-collected ascovirus isolates by DNA hybridization, host range, and histopathology. *J. Invertebr. Pathol.* **72**:138–146.
 47. Hawtin, R. F., T. Zarkowska, K. Arnold, C. J. Thomas, G. W. Gooday, L. A. King, J. A. Kuzio, and R. D. Possee. 1997. Liquefaction of *Autographa californica* nucleopolyhedrovirus-infected insects is dependent on the integrity of virus-encoded chitinase and cathepsin genes. *Virology* **24**:243–253.
 48. He, F., B. J. Fenner, A. K. Godwin, and J. Kwang. 2006. White spot syndrome virus open reading frame 222 encodes a viral E3 ubiquitin ligase and mediates degradation of host tumor suppressor via ubiquitination. *J. Virol.* **80**:3884–3892.
 49. Heath, C. M., M. Windsor, and T. Wileman. 2001. Aggregosomes resembles sites specialized for virus assembly. *J. Cell Biol.* **153**:449–455.
 50. Herrmann, H., and U. Aebi. 2004. Intermediate filaments: molecular structure, assembly mechanism, and integration into functionally distinct intracellular scaffolds. *Annu. Rev. Biochem.* **73**:749–789.
 51. Higashi, K., R. Tajasawa, A. Yoshimori, T. Goh, S. Tanuma, and K. Kuchitsu. 2005. Identification of a novel gene family, paralogs of inhibitor of apoptosis proteins present in plants, fungi, and animals. *Apoptosis* **10**:471–480.
 52. Hirabayashi, T., T. Murayama, and T. Shimizu. 2004. Regulatory mechanism and physiological role of cytosolic phospholipase A₂. *Biol. Pharm. Bull.* **27**:1168–1173.
 53. Hirschberg, H. J. H. B., J. W. F. A. Simons, N. Dekker, and M. R. Egmond. 2001. Cloning, expression, purification and characterization of patatin, a novel phospholipase A. *Eur. J. Biochem.* **268**:5037–5044.
 54. Hughes, A. L. 2002. Evolution of inhibitor of apoptosis in baculoviruses and their insect hosts. *Infect. Genet. Evol.* **2**:3–10.
 55. Ikeda, M., K. Yanagimoto, and M. Kobayashi. 2004. Identification and functional analysis of *Hypantia cunea* nucleopolyhedrovirus *iap* genes. *Virology* **321**:359–371.
 56. Iyer, L. M., S. Balaji, E. V. Koonin, and L. Aravind. 2006. Evolutionary genomics of nucleocytoplasmic viruses. *Virus Res.* **111**:156–184.
 57. Iyer, L. M., E. V. Koonin, D. D. Leipe, and L. Aravind. 2005. Origin and evolution of the archaeo-eukaryotic primase superfamily and related palm-

- domain proteins: structural insights and new members. *Nucleic Acids Res.* 33:3875–3896.
58. Jakob, N. J., K. Muller, U. Bahr, and G. Darai. 2001. Analysis of the first complete DNA sequence of an invertebrate iridovirus: coding strategy of the genome of chilo iridescent virus. *Virology* 286:182–196.
 59. Jakobsson, A., R. Westerberg, and A. Jacobsson. 2006. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog. Lipid Res.* 45:237–249.
 60. Jayadev, S., H. L. Hayter, N. Andrieu, C. J. Gamard, B. Liu, R. Balu, M. Hayakawa, F. Ito, and Y. A. Hannun. 1997. Phospholipase A₂ is necessary for tumor necrosis factor α -induced ceramide generation in L929 cells. *J. Biol. Chem.* 272:17196–17203.
 61. Jehle, J. A., G. W. Blissard, B. C. Bonning, J. S. Cory, E. A. Herniou, G. F. Rohrmann, D. A. Theilmann, S. M. Thiem, and J. M. Vlak. 2006. On the classification and nomenclature of baculoviruses: a proposal for revision. *Arch. Virol.* 151:1257–1266.
 62. Kamita, S. G., A. C. Hinton, C. E. Wheelock, M. D. Wogulis, D. K. Wilson, N. M. Wolf, J. E. Stok, B. Hock, and B. D. Hammock. 2003. Juvenile hormone (JH) esterase: why are you so JH specific? *Insect Biochem. Mol. Biol.* 22:1261–1273.
 63. Kang, W. K., N. Imai, M. Suzuki, M. Iwanaga, S. Matsumoto, and E. A. Zemskov. 2003. Interaction of *Bombyx mori* nucleopolyhedrovirus BRO-A and host cell protein laminin. *Arch. Virol.* 148:99–113.
 64. Kline-Smith, S. L., S. Sandall, and A. Desai. 2005. Kinetocore-spindle microtubule interactions during mitosis. *Curr. Opin. Cell Biol.* 17:35–46.
 65. Kolesnick, R., and Y. A. Hannun. 1999. Ceramide and apoptosis. *Trends Biochem. Sci.* 24:224–227.
 66. Kreuze, J. F., E. I. Savenkov, W. Cuellar, X. Li, and J. P. Valkonen. 2005. Viral class 1 RNase III involved in suppression of RNA silencing. *J. Virol.* 79:7227–7238.
 67. Leipe, D. D., L. Aravind, and E. V. Koonin. 1999. Did DNA replication evolve twice independently? *Nucleic Acids Res.* 27:3389–3401.
 68. Liang, Y., and B. Roizman. 2006. State and role of SRC family kinases in replication of herpes simplex virus 1. *J. Virol.* 80:3349–3359.
 69. Lin, H. W., Y. Y. Chang, M. L. Wong, J. W. Lin, and T. J. Chang. 2004. Function analysis of virion host shutoff protein of pseudorabies virus. *Virology* 324:412–418.
 70. Ling, M., S. W. Allen, and J. M. Wood. 1994. Sequence analysis identifies the proline dehydrogenase and delta 1-pyrroline-5-carboxylate dehydrogenase domains of the multifunctional *Escherichia coli* PutA protein. *J. Mol. Biol.* 243:950–956.
 71. Liu, Y., G. L. Borchert, S. P. Donald, A. Surazynski, C. A. Hu, C. J. Weydert, L. W. Oberley, and J. M. Phang. 2005. MnSOD inhibits proline oxidase-induced apoptosis in colorectal cancer cells. *Carcinogenesis* 26:1335–1342.
 72. Lu, L., S. Y. Zhou, C. Chen, S. P. Weng, S. M. Chan, and J. G. He. 2005. Complete genome sequence of an iridovirus isolated from the orange-spotted grouper, *Epinephelus coioides*. *Virology* 339:81–100.
 73. Meister, R. J., H. Oldenhof, J. L. Bowman, and C. S. Gasser. 2005. Multiple protein regions contribute to differential activities of YABBY proteins in reproductive development. *Plant Physiol.* 137:651–662.
 74. Munter, S., M. Way, and F. Frischknecht. 2006. Signaling during pathogen infection. *Sci. STKE* 335:re5.
 75. Muro, I., J. C. Means, and R. J. Clem. 2005. Cleavage of the apoptosis inhibitor DIAP1 by the apical caspase DRONC in both normal and apoptotic *Drosophila* cells. *J. Biol. Chem.* 280:18683–18688.
 76. Murti, K. G., and R. Goorha. 1983. Interaction of frog virus-3 with the cytoskeleton. 1. Altered organization of microtubules, intermediate filaments, and microfilaments. *J. Cell Biol.* 96:1248–1257.
 77. Nandi, D., P. Tahiliani, A. Kumar, and D. Chandu. 2006. The ubiquitin-proteasome system. *J. Biosci.* 31:137–155.
 78. Nasmyth, K., and C. H. Haering. 2005. The structure and function of SMC and kleisin complexes. *Annu. Rev. Biochem.* 74:595–648.
 79. Nicholson, A. W. 2003. The ribonuclease III superfamily: forms and function in RNA maturation, decay, and gene silencing, p. 149–174. In G. Hannon (ed.), *RNAi: a guide to gene silencing*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
 80. Okano, K., A. L. Vanarsdall, V. S. Mikhailov, and G. F. Rohrmann. 2006. Conserved molecular systems of the *Baculoviridae*. *Virology* 344:77–87.
 81. O'Reilly, D. R., A. M. Crawford, and L. K. Miller. 1989. Viral proliferating cell nuclear antigen. *Nature* 337:606.
 82. Ramanadhan, S., F. F. Hus, S. Zhang, C. Jin, A. Bohrer, H. Song, S. Bao, Z. Ma, and J. Turk. 2004. Apoptosis of insulin-secreting cells induced by endoplasmic reticulum stress is amplified by overexpression of group VIA calcium-independent phospholipase A2 (iPLA2B) and suppressed by inhibition of iPLA2B. *Biochemistry* 43:918–930.
 83. Rydel, T. J., J. M. Williams, E. Krieger, F. Moshiri, W. C. Stallings, S. M. Brown, J. C. Pershing, J. P. Purcell, and M. F. Alibhai. 2003. The crystal structure, mutagenesis, and activity studies reveal that patatin is a lipid acyl hydrolase with a ser-asp catalytic dyad. *Biochemistry* 42:6696–6708.
 84. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463–5467.
 85. Satoh, T., and M. Hosokawa. 1998. The mammalian carboxylesterases: from molecules to functions. *Annu. Rev. Pharmacol. Toxicol.* 38:257–288.
 86. Senkevich, T. G., E. V. Koonin, J. J. Bugert, G. Darai, and B. Moss. 1997. The genome of molluscum contagiosum virus: analysis and comparison with other poxviruses. *Virology* 233:19–42.
 87. Senkevich, T. G., C. L. White, E. V. Koonin, and B. Moss. 2002. Complete pathway for protein disulfide bond formation encoded by poxvirus. *Proc. Natl. Acad. Sci. USA* 99:6667–6672.
 88. Senkevich, T. G., C. L. White, A. Weisberg, J. A. Granek, E. J. Wolffe, E. V. Koonin, and B. Moss. 2002. Expression of the vaccinia virus A2.5L redox protein is required for virion morphogenesis. *Virology* 300:296–303.
 89. Shinzawa, K., and Y. Tsujimoto. 2003. PLA₂ activity is required for nuclear shrinkage in caspase-independent cell death. *J. Cell Biol.* 163:1219–1230.
 90. Shuman, S. 1991. Site-specific interaction of vaccinia virus topoisomerase I with duplex DNA. *J. Biol. Chem.* 266:11372–11379.
 91. Siskind, L. J. 2005. Mitochondrial ceramide and the induction of apoptosis. *J. Bioenerg. Biomembr.* 37:143–153.
 92. Slack, J. M., J. Kuzio, and P. Faulkner. 1995. Characterization of ν -cath, a cathepsin L-like proteinase expressed by the baculovirus *Autographa californica* nuclear polyhedrosis virus. *J. Gen. Virol.* 76:1091–1098.
 93. Smiley, J. R. 2004. Herpes simplex virus virion host shutoff protein: immune evasion mediated by viral RNase? *J. Virol.* 78:1063–1068.
 94. Smith, G. R. 2001. Homologous recombination near and far from DNA breaks: alternative roles and contrasting views. *Annu. Rev. Genet.* 25:243–274.
 95. Sriskanda, V., and S. Shuman. 1998. Specificity and fidelity of strand joining by *Chlorella* virus DNA ligase. *Nucleic Acids Res.* 26:3536–3541.
 96. Starita, L. M., and J. D. Parvin. 2006. Substrates of the BRCA1-dependent ubiquitin ligase. *Cancer Biol. Ther.* 5:137–141.
 97. Stasiak, K., M. V. Demattei, B. A. Federici, and Y. Bigot. 2000. Phylogenetic position of the *Diadromus pulchellus* ascovirus DNA polymerase among viruses with large double-stranded DNA genomes. *J. Gen. Virol.* 81:3059–3072.
 98. Stasiak, K., S. Renault, M. V. Demattei, Y. Bigot, and B. A. Federici. 2003. Evidence for the evolution of ascoviruses from iridoviruses. *J. Gen. Virol.* 84:2999–3009.
 99. Stasiak, K., S. Renault, B. A. Federici, and Y. Bigot. 2005. Characteristics of pathogenic and mutualistic relationships of ascoviruses in field populations of parasitoid wasps. *J. Insect Physiol.* 51:103–115.
 100. Strickland, J. A., G. L. Orr, and T. A. Walsh. 1995. Inhibition of *Diabrotica* larval growth by patatin, the lipid acylhydrolase from potato tubers. *Plant Physiol.* 109:667–674.
 101. Suikkanen, S., M. Antila, A. Jaatinen, M. Vihinen-Ranta, and M. Vuento. 2003. Release of canine provirus from endocytic vesicles. *Virology* 316:267–280.
 102. Tian, M., D. A. Jones, M. Smith, R. Shinkura, and F. W. Alt. 2004. Deficiency in nuclease activity of xeroderma pigmentosum G in mice leads to hypersensitivity to UV irradiation. *Mol. Cell. Biol.* 24:2237–2242.
 103. Tillman, P. G., E. L. Styer, and J. J. Hamm. 2004. Transmission of an ascovirus from *Heliothis virescens* (Lepidoptera, Noctuidae) and effects of the pathogen on survival of a parasitoid, *Cardiochiles nigricipes* (Hymenoptera, Brachonidae). *Environ. Entomol.* 33:633–643.
 104. Traktman, P., and K. Boyle. 2004. Methods for analysis of poxvirus DNA replication. *Methods Mol. Biol.* 269:169–186.
 105. Vaux, D. L., and J. Silke. 2005. IAPs, RINGs and ubiquitylation. *Nat. Rev. Mol. Cell Biol.* 6:287–297.
 106. Visse, R., and H. Nagase. 2003. Matrix metalloproteinases and tissue inhibitors of metalloproteinase: structure, function, and biochemistry. *Circ. Res.* 92:827–839.
 107. Wang, L., J. Xue, C. P. Seaborn, B. M. Arif, and X. W. Cheng. 2006. Sequence and organization of the *Trichoplusia ni* ascovirus 2c (Ascoviridae) genome. *Virology* 354:167–177.
 108. White, C. L., T. G. Senkevich, and B. Moss. 2002. Vaccinia virus G4L glutaredoxin is an essential intermediate of a cytoplasmic disulfide bond pathway required for virion assembly. *J. Virol.* 76:467–472.
 109. Zemskov, E. A., W. Kang, and S. Maeda. 2000. Evidence for nucleic acid binding and nucleosome association of *Bombyx mori* nucleopolyhedrovirus BRO proteins. *J. Virol.* 74:6784–6789.
 110. Zhang, H., F. A. Kolb, L. Jaskiewicz, E. Westhof, and W. Filipowicz. 2004. Single processing center models for human Dicer and bacterial RNase III. *Cell* 118:57–68.
 111. Zhang, Y., I. Calm-Jageman, J. R. Gurnon, T. J. Choi, B. Adams, A. W. Nicholson, and J. L. Van Etten. 2003. Characterization of a *Chlorella* virus PBCV-1 encoded ribonuclease III. *Virology* 317:73–83.
 112. Zhao, S., X. Y. Du, M. Q. Chai, J. S. Chen, Y. C. Zhou, and J. G. Song. 2002. Secretory phospholipase A₂ induces apoptosis via a mechanism involving ceramide generation. *Biochim. Biophys. Acta* 1581:75–88.