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Baculovirus Enhancins and Their Role in Viral Pathogenicity

James M. Slavicek
USDA Forest Service
USA

1. Introduction

Baculoviruses are a large group of viruses pathogenic to arthropods, primarily insects from the order Lepidoptera and also insects in the orders Hymenoptera and Diptera (Moscardi 1999; Herniou & Jehle, 2007). Baculoviruses have been used to control insect pests on agricultural crops and forests around the world (Moscardi, 1999; Szewczk et al., 2006, 2009; Erlandson 2008). Efforts have been ongoing for the last two decades to develop strains of baculoviruses with greater potency or other attributes to decrease the cost of their use through a lower cost of production or application. Early efforts focused on the insertion of foreign genes into the genomes of baculoviruses that would increase viral killing speed for use to control agricultural insect pests (Black et al., 1997; Bonning & Hammock, 1996). More recently, research efforts have focused on viral genes that are involved in the initial and early processes of infection and host factors that impede successful infection (Rohrmann, 2011). The enhancins are proteins produced by some baculoviruses that are involved in one of the earliest events of host infection. This article provides a review of baculovirus enhancins and their role in the earliest phases of viral infection.

2. Lepidopteran specific baculoviruses

The *Baculoviridae* are divided into four genera: the Alphabaculovirus (lepidopteran-specific nucleopolyhedroviruses, NPV), Betabaculovirus (lepidopteran specific Granuloviruses, GV), Gammabaculovirus (hymenopteran-specific NPV), and Deltabaculovirus (dipteran-specific NPV) (Jehle et al., 2006). Baculoviruses are arthropod-specific viruses with rod-shaped nucleocapsids ranging in size from 30-60 nm x 250-300 nm. Most baculoviruses produce two types of virus particles, the occlusion-derived virion (ODV) and the budded virion (BV). ODV are enclosed in a paracrystalline protein matrix, termed the occlusion body (OB) produced by NPVs and the granule produced by GVs, which are composed primarily of the proteins polyhedrin and granulin, respectively. The OB/granule provides the embedded ODV protection from environmental elements such UV light, rain, etc. The BV is produced in the early stages of viral replication and spreads the infection throughout the susceptible cells within the host (Rohrmann, 2011). The NPVs produce OBs that range in size from 0.15 to 3 μm in size and contain many virions. The GVs generate smaller ovoid shaped granules of 0.13 – 0.5 μm that contain a single virion (Ackermann & Smirnoff, 1983). Baculovirus genomes are circular double-stranded DNA molecules ranging in length from about 80 -180

kbp and contain from approximately 90 to 185 open reading frames (ORFs). All baculoviruses sequenced to date contain unique and common ORFs. More than 800 orthologous gene groups have been identified as a consequence of speciation events during viral evolution (Jehle, et. al., 2006). Baculovirus genomes undergo a high rate of mutation as a consequence of gene duplication and loss, homologous recombination, and gene transfer from other viruses, bacteria, and eukaryotic genomes. Sequence analysis of NPV and GV genomes revealed that the NPVs and GVs contain many gene homologs, and 29 core genes are conserved in all baculoviruses sequenced to date (Herniou et al., 2003; Jehle et al., 2006; Herniou & Jehle, 2007). Several groups of genes are conserved in some but not all NPVs and GVs. One of these groups is termed auxiliary genes, which include *enhancin* genes. These genes are not essential for viral DNA replication but provide a selective advantage to a virus (Miller, 1997).

3. Viral pathogenesis

GVs and NPVs initiate infection when a susceptible host ingests viral OBs present on host plants. Within the alkaline environment of the larval midgut, OBs dissolve, thereby releasing ODV. ODV must first traverse a physical structure termed a peritrophic matrix (PM), which is composed of proteins, mucopolysaccharides, and chitin (Pritchett et al., 1984; Hegedus, et al., 2009). The PM provides a barrier to gut cells from bacteria, viruses, fungi, and physical damage from ingested plant material (Lehane, 1997; Terra, 2001). The PM is in a constant state of regeneration from epithelial cells as the larvae feeds. The movement of food material through the insect gut causes loss of the PM.

ODV then gains entry into midgut cells by a type of fusion process (Granados, 1980; Granados & Lawler, 1981) that is not defined. The type NPV, *Autographa californica* multiple NPV (AcMNPV), initiates the infection cycle by infecting primarily columnar epithelial cells within the midgut and to a much lesser extent regenerative epithelial cells in *Trichoplusia ni* (Keddie et al., 1989) or *Spodoptera exigua* larvae (Flipsen et al., 1995). Several factors are involved during the initial act of infection that includes ODV binding to midgut cells at cell receptors and viral entry into the cells. All sequenced baculoviruses contain genes that code for *per os* infectivity factors (PIFs) that are associated with ODVs but not BV (Faulkner et al., 1997; Kikhno et al., 2002; Fang et al., 2006; Harrison et al., 2010; Fang et al., 2009). The *pif* genes include p74-pif, and *pif* genes 1-5, Ac119, Ac 22, Ac115, Ac96, and Ac148, respectively. Deletion of any of the *pif* genes from a viral genome significantly decreases but does not eliminate *per os* infectivity (d'Alencon et al., 2004; Crouch et al., 2007). The PIFs, with the exception of PIF3, are thought to be involved in binding or interacting with the midgut cells that leads to infection (Ohkawa, et al., 2005; Li et al., 2007; Peng et al., 2010; Horton & Burand, 1993). Another gene present in some GVs and NPVs, the *enhancin* gene, codes for a protein that also impacts viral potency during *per os* infection as described in the next section.

Upon entry into midgut cells, the nucleocapsids are actively transported to nuclear pores in a process that uses actin polymerization (Ohkawa et al., 2010). Viral DNA is then released into the cell nucleus and viral replication ensues (Rohrmann, 2011). During the early phase of viral replication, BV are produced that bud from midgut cells and infect tracheal epidermal cells, which penetrate the basal lamina (Volkman, 2007). Infection spreads via the tracheal system and haemocytes until many different cell types are infected (Engelhard et

al., 1994). During the later phase of viral replication, ODV are produced and packaged within OBs. Upon the host's death, liquefaction occurs, releasing OBs into the environment that can lead to infection of another host.

4. Early studies on the "synergistic factor" in Granuloviruses

Early studies with GVs identified a factor in the *Pseudaletia unipuncta* (Psun) GV that increased the infectivity of PsunNPV in NPV/GV mixed infections (Tanada, 1959). Early *P. unipuncta* instars were highly susceptible to PsunNPV and PsunGV, whereas the later instars (4-6) were increasingly less susceptible. In contrast, when larvae were infected *per os* with both NPV and GV viruses, they were highly susceptible. Feeding of larvae first with PsunNPV, followed by PsunGV did not generate synergy, whereas synergy was observed when the larvae were first infected with PsunGV followed by PsunNPV indicating the synergistic factor was associated with PsunGV. The results of heat-inactivation experiments indicated that a component within the GV ODV envelope or the granule was responsible for the synergism (Tanada, 1959). The enhancing factor was named the synergistic factor (SF), and was found to be a protein component of the GV capsule (Hara et al., 1976; Tanada et al., 1973), comprising about 5% of the capsule protein components (Yamamoto & Tanada, 1978). When purified SF was added to PsunNPV ODV it exhibited a strong affinity for viral envelopes, and with about 8 molecules of SF bound to each enveloped virion the infectivity of the ODV were significantly enhanced (Yamamoto & Tanada, 1980). A synonymous factor, (viral enhancing factor, VEF) was found in *Trichoplusia ni* GV (TnGV) and *Xestia c-nigrum* GV (XecnGV) granules, which enhanced the infectivity of AcNPV, and *Xestia c-nigrum* NPV (XecnNPV), respectively (Derksen & Granados, 1988; Gallo et al., 1991; Goto et al., 1990).

Initial studies on the function of enhancins suggested that the site of SF action is the cellular membrane of the midgut cell microvilli (Tanada et al., 1975; Tanada et al., 1980), and these cells contain specific binding sites for enhancins (Uchima et al., 1988). Electron microscopy was used to visualize and count attached virions on midgut epithelium in *P. unipuncta* larvae. Electron micrographs of midguts treated with PsunNPV and SF exhibited 40 times more nucleocapsids attached or within microvilli compared to midguts treated only with PsunNPV (Tanada et al., 1975). In addition, antibody studies also localized the site of SF binding to midgut cell membranes (Tanada et al., 1980). Competition binding studies with Concanavalin A and castor bean lectin were found to inhibit binding of SF to midgut cell membranes, suggesting that SF binding was to specific receptors (Uchima et al., 1988).

Subsequent studies by Granados and colleagues demonstrated that the enhancin from TnGV degraded major glycoproteins of 123, 194, and 253 kDa within the PM (Derksen and Granados, 1988). In addition, the PMs of virus treated larvae were fragile compared to controls suggesting a physical weakening of the PM structure had occurred. Purified VEF from TnGV was found to significantly increase infectivity of AcMNPV in *T. ni* larvae in a linear dose-dependent manner. The major effect of VEF treatment appeared to be the disruption of the PM, which is the likely basis for increased NPV potency (Gallo et al., 1991).

4.1 Identification and analysis of baculovirus *enhancin* genes

The first gene encoding a VEF was identified in TnGV and sequenced (Hashimoto et al., 1991). Subsequent studies identified *enhancin* genes in several GVs and a few NPVs as listed in Table

1. *Enhancin* genes have been identified in approximately 30% of NPVs and GVs sequenced to date. These genes are more common in GVs, present in about 46% of the genomes analyzed to date vs. approximately 24% of NPVs (Table 1). Several GVs and NPVs contain multiple *enhancin* genes, and the XecnGV contains the most at four. In addition to baculoviruses, *enhancin* genes have been identified in the genomes of microorganisms including *Bacillus cereus* (Ivanova et al., 2003), *Bacillus anthracis* (Read et al., 2003), *Bacillus thuringiensis* (accession no. NC 005957), *Yersinia pestis* (Parkhill et al., 2001), *Salmonella enterica* subsp. enterica serovar Javiana strain GA_MM04042433 (accession no. NZ_ABEH02000001, *Clostridium perfringens* D strain JGS1721 (accession no. ZP_02954459), *Aspergillus oryzae* RIB40 (accession no. XM_001817293), *Enterobacter aerogenes* KCTC 2190 (accession no. NC_015663), and *Listeria ivanovii* subspecies ivanovii PAM 55 (accession no. NC_016011).

Virus	Host	# Enhancin Genes Present	Accession Number[c]
AcMNPV	*Autographa californica*	__	NC_001623
AdhoNPV	*Adoxophyes honmai*	__	NC_004690
AdorGV	*Adoxophyes orana*	__	NC_005038
AgipMNPV	*Agrotis ipsilon*	1	NC_011345
AgseGV	*Agrotis segetum*	1	NC_005839
AgseNPV	*Agrotis segetum*	3	NC_007921
AgMNPV	*Anticarsia gemmatalis*	__	NC_008520
AnpeNPV	*Antheracea pernyi*	__	NC_008035
BmNPV	*Bombyx mori*	__	NC_001962
ChchNPV	*Chrysodeixis chalcites*	__	NC_007151
ChocGV	*Choristoneura occidentalis*	__	NC_008168
CfMNPV	*Choristoneura fumiferana*	1	NC_004778
CfDefNPV	*Choristoneura fumiferana*	__	NC_005137
CfGV[a]	*Choristoneura fumiferana*	1	AF319939
ClbiNPV	*Clanis bilineata*	__	NC_008293
CpGV	*Cydia pomonella*	__	NC_002816
CuniNPV	*Culex nigripalpus*	__	NC_003084
CrleGV	*Cryptophlebia leucotreta*	__	AY_009987
EcobNPV	*Ecotroplis obliqua*	__	NC_008586
EppoNPV	*Epiphyas postvittana*	__	NC_003083
EupsNPV	*Euproctis pseudoconspersa*	1	NC_012693
HearGV	*Helicoverpa armigera*	4	NC_010240
HearMNPV	*Helicoverpa armigera*	1	NC_011615
HearSNPV	*Helicoverpa armigera*	__	NC_002654
HycuNPV	*Hyphantria cunea*	__	NC_007767
HzSNPV	*Helicoverpa zea*	__	NC_003349
LdMNPV	*Lymantria dispar*	2	NC_001973
LyxyMNPV	*Lymantria xylina*	2	NC_013953
LeseNPV	*Leucanua separata*	__	NC_008348
MacoNPV-A	*Mamestra configurata*	1	NC_003529
MacoNPV-B	*Mamestra configurata*	1	NC_004117
MaviNPV	*Maruca vitrata*	__	NC_008725
NeabNPV	*Neodiprion abietis*	__	NC_008252

NeleNPV	*Neodiprion lecontei*	__	NC_005906
NeseNPV	*Neodiprion sertifer*	__	NC_005905
OpMNPV	*Orgyia pseudotsugata*	__	NC_001875
OrleNPV	*Orgyia leucostigma*	__	NC_010276
PhopGV	*Phthorimaea operculella*	__	NC_004062
PlxyMNPV-CL3	*Plutella xylostella*	__	DQ_457003
PlxyGV	*Plutella xylostella*	__	NC_002593
PsunGV	*Pseudaletia unipuncta*	3[a]	NC_013772
RoMNPV	*Rachiplusia ou*	__	NC_004323
SeMNPV	*Spodoptera exigua*	__	NC_002169
SfMNPV	*Spodoptera frugiperda*	__	NC_009011
SpltGV	*Spodoptera litura*	__	NC_009503
SpltMNPV	*Spodoptera litura*	__	NC_003102
TnSNPV	*Trichoplusia ni*	__	NC_007383
TnGV	*Trichoplusia ni*	1[b]	D12617
XcGV	*Xestia c-nigrum*	4	NC_002331

The genus and species of the host organism from which the virus was isolated were used to name the virus using either the first letter of the host genus and species, or the first two letters. NPV stands for nucleopolyhedrovirus, GV for granulovirus, M for multiply enveloped ODV, and S for singly enveloped ODV.

[a] PsunGV does not contain a homologue of HearGV VEF-2 and XecnGV VEF-2 genes. The sequence of the PsunGV VEF-1 sequenced by Roelvink et al., (1995) matches the sequence of Psun VEF-3 reported in the genomic sequence.

[b] The sequence listed for is for only the *enhancin* gene, the genome of this virus has not been sequenced.

[c]References for the accession numbers are AcMNPV, Ayres et al., 1994; AdhoNPV, Nakai et al., 2003; AdorGV, Wormleaton et al., 2003; AgipMNPV, Harrison, 2009; AgseMNPV, Jakubowska et al., 2006; AgMNPV, Oliveira et al., 2006; AnpeMNPV, Fan et al., 2007; BmNPV, Gomi et al., 1999; ChchNPV, van Oers et al., 2005; ChocGV, Escasa et al., 2006; CfMNV, de Jong et al., 2005; CfDefNPV, Lauzon et al., 2005; ClbiNPV, Zhu, S., et al., 2009; CpGV, Luque et al., 2001; CuniNPV, Afonso et al., 2001; CrleGV, Lange & Jehle, 2003; EcobNPV, Ma et al., 2007; EppoNPV, Hyink et al., 2002; EupsNPV, Tang et al., 2009; HearGV, Harrison & Popham, 2008; HearSNPV G4, Chen et al., 2001; HycuNPV, Ikeda et al., 2006; HzSMPV, Chen et al., 2002; LdMNPV, Kuzio et al., 1999; LyxyMNPV, Nai et al., 2010; LeseNPV, Xiao & Qi, 2007; MacoNPV-A, Li, Q., etal., 2002; MacoNPV-B, Li, L., etal., 2002; MaviNPV, Chen et al., 2008; NeabNPV, Duffy, et al., 2006; NeleNPV, Lauzon, et al., 2004; NeseNPV, Garcia-Maruniak, et al., 2004; OpMNPV, Ahrens, et al., 1997; PlxyGV, Hashimoto, et al., 2000; PlxyMNPV-CL3, Harrison & Lynn, 2007; RoMNPV, Harrison & Bonning, 2003; SeMNPV, Ijkel et al., 1999; SfMNPV, Harrison et al., 2008; SpltGV, Wang et al., 2008; SpltMNPV, Pang et al., 2001; TnSNPV, Willis et al., 2005; XcGV, Goto et al., 1998. Sequences only submitted to GenBank: AgseGV, CfGV, HearMNPV, OrleNPV, PhopGV, PsunGV.

Table 1. Sequenced Baculovirus Genomes and Genbank Accession Numbers

Comparison of the LdMNPV-VEF-1 enhancin amino acid sequence with sequences in the BLOCKS database (version 9.0, December 1995 [Henikoff & Henikoff, 1991]) revealed the presence of a signature pattern characteristic of a zinc-binding domain found within metalloproteases (Jongeneel et al., 1989; Murphy et al., 1991). The signature pattern, HEXXH, is sufficient to group a protein into the metalloprotease superfamily. Most baculovirus enhancins have this conserved metalloprotease zinc-binding domain (residues 241 to 246 for the Ld*M*NPV-VEF-1) (Table 2). For this type of enzyme, the zinc ion is chelated by the two histidine residues in this sequence and by a third residue, typically a histidine, cysteine, or aspartic or glutamic acid residue, located anywhere from 20 to 120 aa

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LdMNPV-VEF-1      VGAAYYDRNWTAQTNVSMS-RYLQPRATNWLVL  HEIGH  AYDFQFVS--NTPALNEVWNNV 266
LyxyMNPV-VEF-1    VGVAYYGREWTAQTNVSMS-RYLQPRATNWLVL  HEIGH  AYDFQFVI--NTPSLIEVWNNV 266
LyxyNPV-VEF-2       AGIAFYGQHWLGASTNTLL-RYLNVDADPWLIL  HEIGH  GHEFAFVG--TAPPLSEVWTNI 268
LdMNPV-VEF-2        AGIAFYGQHWIGASANTLL-RYLNVDADPWLIL  HEIGH  GHEFAFVG--TAPPLIEVWTNI 268
EupsNPV           SGIAYYGTFWIAHSSYSIS-EYLKADPTNWLAL  HEVGH  AYDFNFVT--NVPVLSEVWTNV 276
XecnGV-VEF-4     HGAAYYSSKWLAMSQSDLS-FFLVPSYTNWLVL  QKLGD  AYNFGFTR--EHTYMAGAWSGM 259
HearGV-VEF-4     HGAAYYSSNWLAMSQSDLS-YFLVPSYTNWLVL  QTLGD  AYNFGFTR--EHTYWAGAWSGM 259
PsunGV-VEF-4     RDTAYYSSNWLAMSQSDLG-KFLEPSFTNWLVL  QTLGD  AYNYGFTR--YDTYWLGVWSGM 259
HearGV-VEF-3     PGGAYYGAFWTAPASTNLG-EYLRVSPTNWMVI  HELGH  AYDFVFT---VNTRLIEIWNNS 267
XecnGV-VEF-3     PGGAYYGPFWTAPANTSLR-DYLVVSPTNWMVI  HELGH  AYDFVFT---VNTRLIEIWNNS 267
PsunGV-VEF-3     PGGAYYGPFWTAPASSNLG-DYLRISPTNWMVI  HELGH  AYDFVFT---VNTILIEIWNNS 267
CfGV            PGGAYYGPFWTAPASSNLG-DYLRISPTNWMVI  HELGH  AYDFVFT---VNTILIEIWNNS 267
TnGV            PGGAYYGPFWTAPASSNLG-DYLRISPTNWMVI  HELGH  AYDFVFT---VNTILIEIWNNS 267
XecnGV-VEF-2     PGAAYYGGSWTANSQSVLG-NYLQVRPGNWLVF  HEIGH  AYDLVFT---QGTNLHEVWNNV 270
HearGV-VEF-2     PGAAYYGGSWTANSQSVLG-NYLQVRPGNWLVF  HEIGH  AYDLVFT---QGTNLHEVWNNV 270
XecnGV-VEF-1     PAEHYYTDAYIANSADTLG-PFLLSTITNWPAL  HQIGH  GYDFNFT---NHTVLIEVWSSV 251
HearGV-VEF-1     PAEHYYTDAYIANSADTLG-PFLLSTITNWPAL  HQIGH  GYDLNFT---NHTVLIEVWSSV 251
PsunGV-VEF-1     PGDHYYSDSYIANSANTLG-PFLLSTITNWPAL  HQIGH  GYDHHFT---NNTSLKEVWSSV 252
AgseNPV-VEF-2    PGDAFYHTWYMGESYETMKSFYLTPSTLNWGAL  HEMAH  SFDIYFARNTIQVSLQEVWTNI 258
AgipMNPV        GGGAFYGHYFMGASSQSMARFFLNLTPLNWGGL  HEIGH  SFDLVFTRNTSQLDVQEVWTNV 256
AgseNPV-VEF-1    PGGAYYSRLYMGESSASMRSFYLRPTPLNWGCL  HEISH  SLDIYFRHNSEQVSLVEVWTNI 254
MacoNPV-B       VGGAYYGKYTMAESSPSMRRFYLTPSKFNWGCL  HEIAH  SFDAHFTSNYIHADIREVWTNI 254
HearMNPV        VGGAYYGKYTMAESSPSMRRFYLTPSKFNWGCL  HEIAH  SFDAHFTSNYIHADIREVWTNI 254
MacoNPV-A       AGGGYYGKYTMGESNPSMRRFYLTPSKFNWGCL  HEIAH  SFDAYFTWNYAHADIREVWTNI 254
AgseGV          SGAAYYGPFWIGVTTQSFD-LFYNVSISNWIML  HVMGH  AYDFEFAN--SKSIFEETWASI 271
CfMNPV          SGAAYYNNLTMGQSNSSVEHFYLRPLPTNWGGL  HEIAH  AYDFHFVRS-GPVPLNEVWNNI 265

LdMNPV-VEF-1      LADRYQYDFMSFDERQRDASVYENGNRDRVERNIAERIDNRAPY--ESWSFFQKMAVFTW 324
LyxyMNPV-VEF-1    LADRYQYDFMSFDERQRDASVYENGNRDRVERNIAELIRNRVPY--ENWSFFQKLTVFTW 324
LyxyNPV-VEF-2     LPNLFQFDTMTSAERETAAWIYDYGRREAVERGLGALIDQRVDY--DKFSFRERLFAYAP 326
LdMNPV-VEF-2      LPNLFQFDTMTSAERQTAAWIYDHGRREVVERGLGALIDQRVDY--DKFSFRERLFAYAP 326
EupsNPV         LPDRYQFYNMTYEERQRLSWIFGEGNRQTVEKSLQSLISSKTKYSSDDYYFRERLLTLGL 336
XecnGV-VEF-4      LSDRLQYYHQTRAERQTVSEIYAG-QRLRVEEEIAAVLLDNLNL--DLWTELHKIVFFTW 316
HearGV-VEF-4      LSDRLQYYHQTRAERQTISEVYAG-QRLRVEEEIAAVLLDNLNL--DLWTELHKIVFFTW 316
PsunGV-VEF-4      LSDRLQYYYQDISERQTLSKIYAG-ERNRIEEEINVTIQDDLNL--DLWTELHKIVFFTW 316
HearGV-VEF-3      FCDRIQYTWMNKTKRQQLARIYEN-QRPQKEAAIQALIDNNVPF--DNWDFFEKLSIFAW 324
XecnGV-VEF-3      FCDRIQYTWMNKTKRQQLARIYEN-QRPQKEAAIQALIDNNVPF--DNWDFFEKLSIFAW 324
PsunGV-VEF-3      LCDRIQYKWMNKTKRQQLARVYEN-RRPQKEATIQALIDNNSPF--DNWGFFERLIIFTW 324
CfGV            LCDRIQYKWMNKTKRQQLARVYEN-RRPQKEATIQALIDNNSPF--DNWGFFERLIIFTW 324
TnGV            LCDRIQYKWMNKIKRQQLARVYEN-RRPQKEATIQALIDNNSPF--DNWGFFERLIIFTW 324
XecnGV-VEF-2      YGDRMQYQLMDAAVRQSSASVYENGNRVRVENNIMNLIDTNVNY--SSWSFFQKLAMFTF 328
HearGV-VEF-2      YGDRMQYQLMDAAVRQSSASVYENGNRVRVENNIMNLIDTNVNY--SSWSFFQKLAMFTF 328
XecnGV-VEF-1      LADSMQYQWMNTAERQKLATIYDGDQREYAETEIVKNLAESASF--NNLDAQWRTILLSF 309
HearGV-VEF-1      LADSMQYQWMNTAERQKLPTIYDGDQREYAETEIVKNLAESASF--NNLDAQWRTILLAF 309
PsunGV-VEF-1      LADSMQYQWMNTTERQNLPMIYDGDQREHAEAEIVKELAESVAF--HDLDTNWRTILLAF 310
AgseNPV-VEF-2      WPDYYQYTRLTTDEYEDKSWMLSP-TREVSIGQLVEKFHTTTLH---DWDHRERLLYLTA 314
AgipMNPV        LPDYYQHTRLSPDEYEANAWLLDP-GREATFGQLIAKFHTTPIH---EWNLRERLIFLTS 312
AgseNPV-VEF-1      FPDFYQYSKLTPAEYQASAWIMGS-SQQTIWGNLIAKFHTVSVH---DWDLRERLIYLVQ 310
MacoNPV-B       MPDYYQYLNYTEEEYLTRGWKYDG-RRDAVLIEIKRIFDVIPFN---KWSLRQRLVFLTS 310
HearMNPV        MPDYYQYLNYTEEEYLTRGWKYDG-RRDAVLIEIKRIFDVIPFN---KWSLRQRLVFLTS 310
MacoNPV-A       MPDYYQYLNFTEEEYLTKSWKLDG-QRDTLFMEIKALFGVVPFN---EWYLRDRLVFLTS 310
AgseGV          FADRYQFFRSTKMERYLTNTVFGPQMYSQIITDINILFSARTPFP--AWPEFRKLLILSL 329
CfMNPV          LCDAYQSRYLELDEKS-----VNSPNSLTMGRNIVRKIENGKVF--DSYNLFEKLAVFSY 318
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[a] The conserved HEXXH sequence is highlighted in turquoise, the non-conserved corresponding sequence in a few enhancins is highlighted in yellow, and aspartic and glutamic residues 20 or more amino acids downstream are highlighted in red and green, respectively.

Table 2. Alignment of Baculovirus Enhancin Proteins with COBALT (NCBI)[a]

downstream of the HEXXH sequence (Häse & Finkelstein, 1993; Jiang & Bond, 1992). There are several aspartic and glutamic acid residues between 20 and 120 aa from the HEXXH sequence that are present in baculovirus enhancins, any of which could function as a third zinc-binding ligand in the enhancin proteins (Table 2). In the metalloproteases, the glutamic acid residue within the HEXXH sequence is the catalytic base, which polarizes a water molecule involved in the nucleophilic attack of the peptide bond to be cleaved. XecnGV-VEF-1, HearGV-VEF-1, PsunGV-VEF-1 contain a glutamine residue in place of the glutamic acid within the HEXXH (HQXXH) consensus, in XecnGV-VEF-4, HearGV-VEF-4, PsunGV-VEF-4 a QXXDG sequence is in place of the consensus sequence, and AgseGV contains a HVMGH sequence in place of the consensus sequence (Table 2). The alterations in the zinc binding domain of these enhancins could have made these proteins non-functional. If so, a virus with a mutated non-functional *enhancin* gene could gain selective advantage through acquisition of a functional *enhancin* gene. These events offer a basis for the presence of multiple *enhancin* genes within viral genomes. A functional analysis of the enhancins in XecnGV-VEF-1, HearGV-VEF-1, PsunGV-VEF-1, XecnGV-VEF-4, HearGV-VEF-4, PsunGV-VEF-4, and AgseGV has not been performed.

A comparison of the number of amino acids in baculovirus enhancins and the proteins that have the most and least amino acid identities are listed in Table 3. Baculovirus enhancins exhibit a great deal of heterogeneity. For example, LdNPV-VEF-1 is 89% identical to LyxyMNPV-VEF-1. In contrast, LdNPV-VEF-1 is only 16% identical to MacoNPV-A (Table 3). The size of baculovirus enhancins is from 758 amino acids for CfMNPV to 1004 amino acids for AgseGV.

A phylogenetic analysis of all currently known baculoviruses was performed using the phylogenetic tree function of CLUSTAL-W, and the tree is shown in Figure 1. All of the GV enhancins, with the exception of AgseGV, form a group, all of the NPV enhancins form a group, and AgseGV is within its own group (Fig 1). Three subgroups are within the GV group; XecnGV-VEF-2 – PsunGV-VEF-1, HearGV-VEF-3 – TnGV, and XecnGV – Psun-VEF-4. Two primary groups are present within the NPV enhancin group; LdMNPV-VEF-1 – LdMNPV-VEF-2, and CfMNPV – MacoNPV-A. The high level of heterogeneity exhibited by the baculovirus enhancins may suggest that these genes arose in viral genomes from independent sources.

The presence of *enhancin* genes in bacteria suggests a possible means for enhancin gene exchange between microorganisms. The *B. cereus* group contains the closely related organisms *B. cereus*, an opportunistic pathogen of humans; *B. anthracis*, a mammalian pathogen; and *B. thuringiensis*, an insect pathogen. The presence of *enhancin* genes in *B. cereus* and *B. anthracis* led to the suggestion that these organisms evolved from an ancestor of the *B. cereus* group that was an opportunistic insect pathogen (Ivanova et al., 2003; Read et al., 2003). The subsequent finding of an *enhancin* gene in *B. thuringiensis* provides further support for this hypothesis. If the *B. cereus* ancestor resided in the guts of insects that were NPV hosts, there may have been an opportunity for an exchange of genetic material between these bacteria and NPVs. The enhancin in *Y. pestis*, the causative agent of the disease referred to as plague, may aid its colonization of the flea. The *Y. pestis enhancin* gene is flanked by a tRNA gene and transposase fragments, which may suggest that this bacteria obtained its *enhancin* gene via horizontal transfer. However, a recent study found that expression of bacterial enhancins in insect cells caused cytotoxicity, and they did not

enhance viral infectivity (Galloway et al., 2005). These results may suggest that bacterial and baculovirus enhancins have evolved different functions.

Viral Enhancin	# of Amino Acids	Viral Enhancin with the Greatest Amino Acid Identity, % Identity	Viral Enhancin with the Least Amino Acid Identity, % Identity
LdMNPV-VEF-1	782	LyxyMNPV-VEF-1, 89%	MacoNPV-A, 16%
LyxyMNPV-VEF-1	782	LdMNPV-VEF-1, 89%	AgseNPV-VRF-2, 17%
EupsNPV	802	LyxyMNPV-VEF-2, 34%	MacoNPV-A, 13%
LyxyMNPV-VEF-2	788	LdMNPV-VEF-2, 94%	MacoNPV-A, 12%
LdMNPV-VEF-2	788	LyxyMNPV-VEF-2, 94%	MacoNPV-B, 12%
XecnGV-VEF-2	867	HearGV-VEF-2, 96%	MacoNPV-B, HearMNPV, AgipMNPV, 13%
HearGV-VEF-2	865	XecnGV-VEF-2, 96%	AgseNPV-VEF-2 AgipMNPV, 13%
HearGV-VEF-3	902	XecnGV-VEF-3, 86%	HearMNPV, 15%
PsunGV-VEF-3	901	TnGV, CfGV, 98%	AgseNPV-VEF-2, 12%
TnGV	901	PsunGV-VEF-3, CfGV, 98%	AgseNPV-VEF-2, 12%
CfGV	901	PsunGV-VEF-3, TnGV, 98%	AgipMNPV, 14%
XecnGV-VEF-3	898	HearGV-VEF-3, 86%	AgipMNPV, 15%
XecnGV-VEF-4	856	HearGV-VEF-4, 95%	AgseNPV-VEF-2, AgipMNPV, 11%
HearGV-VEF-4	856	XecnGV-VEF-4, 95%	AgseNPV-VEF-2, 11%
PsunGV-VEF-4	857	XecnGV-VEF-1, 23%	AgseNPV-VEF-1, 11%
XecnGV-VEF-1	824	HearGV-VEF-1, 96%	AgipMNPV, 10%
HearGV-VEF-1	823	Xecn-VEF-1, 98%	AgipMNPV, 10%
CfMNPV	758	AgseNPV-VEF-2, 19%	PsunGV-VEF-1, 16%
PsunGV-VEF-1	828	AgseGV, 21%	AgipMNPV, 12%
AgseGV	1004	PsunGV, 21%	HearMNPV, 13%
AgseNPV-VEF-1	877	AgseNPV-VEF-2, 43%	PsunGV-VEF-4, 11%
MacoNPV-B	848	HearMNPV, 99%	LdMNPV-VEF-2, 12%
HearMNPV	848	MacoNPV-B, 99%	AgseGV, XecnGV-VEF-2, 13%
AgseNPV-VEF-2	883	AgipMNPV, 53%	Xecn-VEF-4, HearGV-VEF-4, 11%
AgipMNPV	897	AgseNPV-VEF-2, 53%	Xecn-VEF-1, HearGV-VEF-1, 10%
MacoNPV-A	847	HearNPV, 81%	LyxyMNPV-VEF-2, 12%

Table 3. Number of Amino Acids in Baculovirus Enhancins and Identity of the Enhancin Most and Least Similar.

4.2 Function of baculovirus enhancins

Studies on proteins within TnGV granules identified a 98 kDa protein that enhanced AcMNPV potency in bioassays (Gijzen et al., 1995). The enhancin from TnGV granules was purified by gel filtration and ion exchange chromatography and was found to be a metalloprotease based on activity inhibition studies using metal chelators (Lepore et al., 1996). In addition, the *enhancin* gene from TnGV was expressed by a recombinant AcMNPV containing this gene, the enhancin protein was purified, and was found to enhance the infectivity of AcMNPV in larval bioassays, thereby confirming that the *enhancin* gene codes for the enhancing factor in TnGV granules (Lepore et al., 1996).

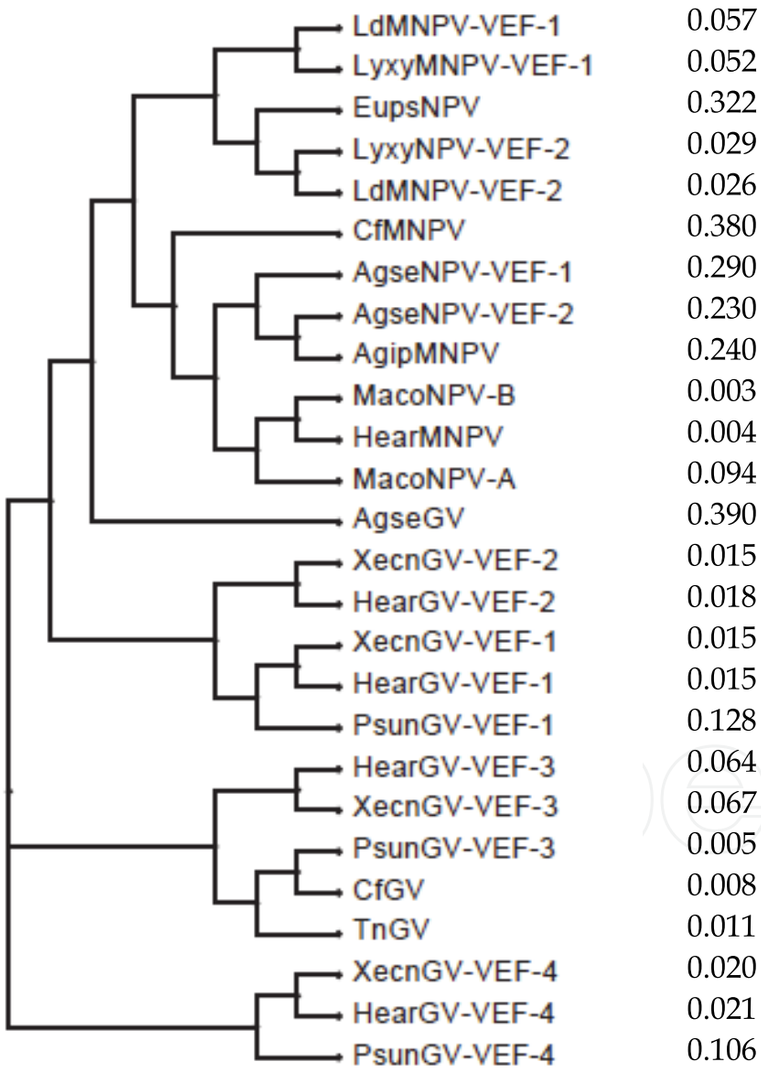

Fig. 1. Phylogenetic tree of NPV and GV enhancins. The distances from the nodes are shown on the right of the figure.

Studies with isolated PMs from *T. ni* and *P. unipuncta* larvae found that PMs treated with purified TnGV enhancin were significantly more permeable to AcMNPV compared to control PMs. These results provide evidence that the PM is a barrier to virus movement across the PM and that TnGV enhancin facilitates infection by altering the permeability of the PM (Peng et al., 1999). Wang and Granados (1997) found that the target substrate for GV enhancins is insect intestinal mucin, a glycosylated protein associated with the PM. Degradation of intestinal mucin increased access of virions to the midgut epithelial cells, increasing susceptibility of the host insect to viral infection (Wang & Granados, 2000). This is likely the basis for the early observations of enhancement of NPV infections *in vivo* when co-administered with GV granules and purified enhancin. Based on these findings, and the finding the enhancins constitute 5% of the protein in GV granules, there is substantial evidence supporting the hypothesis that GV enhancins facilitate GV virus movement through the PM.

However, earlier studies by Tanada and colleagues generated evidence that GV enhancins bind to larval midgut cells at specific sites, and they hypothesized that the enhancin protein serves as a receptor for some NPVs (Tanada, 1985). They found that significantly more virus particles were attached to or already in midgut microvilli in insects treated with enhancin plus PsunNPV compared to insects without enhancin, even though polyhedra and ODV were found in abundance next to microvilli in the control insects. In addition, enhancin was found to be associated with midgut cell microvilli when purified protein was administered *per os* (Tanada et al., 1980), and *P. unipuncta* larval midgut cells were found to contain specific binding sites for GV enhancins that enhance the infection process of NPVs (Uchima *et al.*, 1988). Also, several investigations found that GV enhancins increased infection of NPVs in cell culture systems (Hukuhara & Zhu, 1989; Kozuma & Hukuhara, 1994; Tanada, 1985).

Studies on the function of baculovirus enhancins have been within the context of heterologous systems using GV enhancins/granules to investigate their impact on the potency of NPVs administered *per os*, and the infectivity of NPV BV in cell culture systems. After identification and characterization of the TnGV gene encoding the enhancin protein by Granados and colleagues, studies focused on enhancin biochemical properties and impacts on the PM. However, a direct impact of GV enhancin on GV potency through deletion of the gene from the GV genome has not been demonstrated. Studies on the function of LdMNPV enhancins described below were the first studies performed within a homologous system.

4.3 Analysis of enhancin function(s) in the LdMNPV

The LdMNPV was the first NPV found to contain an *enhancin* gene (Bischoff & Slavicek, 1991), and upon sequencing the viral genome, a second *enhancin* gene was identified (Kuzio et al., 1999). *Enhancin 1* and *2* gene transcripts are expressed at late times after infection from a consensus baculovirus late promoter (Bischoff & Slavicek, 1991; Popham et al., 2001). To investigate the function of the LdMNPV *enhancin 1* gene a recombinant LdMNPV virus was constructed that lacked a functional *enhancin 1* gene (E1cat). Potency analysis through *L. dispar* larval bioassays revealed that the *enhancin 1* gene minus viral strain was

approximately 2-3 fold less potent than wild-type (WT) viruses, suggesting that the LdMNPV enhancin affects viral potency (Bischoff & Slavicek, 1997). The effect of the second LdMNPV *enhancin* gene alone and in conjunction with the *enhancin 1* gene on viral potency was investigated through bioassay using two recombinant viruses, one with a deletion in the *enhancin 2* gene (E2del) and a second with deletion mutations in both *enhancin* genes (E1delE2del) (Popham et al., 2001). The *enhancin* gene viral constructs were verified by Southern analysis and were shown not to produce *enhancin* gene transcripts by Northern analysis. The E2del virus exhibited an average decrease in viral potency of 1.8 fold compared to wild-type virus. In the same bioassays, the recombinant virus E1cat, which does not produce an *enhancin1* gene transcript, exhibited an average decrease in viral potency of 2.3 fold compared to control virus. The E1delE2del virus exhibited an average decrease in viral potency of 12 fold compared to wild-type virus, indicating that the two genes confer a non-additive compensatory enhancement of viral efficacy (Popham *et al*., 2001). Collectively, these results suggest that both LdMNPV *enhancin* genes contribute to viral potency, that each enhancin protein can partially compensate for the lack of the other protein, and that both *enhancin* genes are necessary for wild-type viral potency. Detergent dissociation studies and immuno-electron microscopy were used to localize LdMNPV enhancins in viral structures (Slavicek & Popham, 2005). Polyclonal antibodies specific to LdMNPV enhancin 1 and enhancin 2 identified unique proteins of 84 and 90 kDa, respectively, at 48 h post infection (p.i.) in infected cell extracts prepared from 0 to 96 h p.i. The 84- and 90-kDa proteins are close to the predicted sizes of Enhancin 1 (89.2) and Enhancin 2 (88.4), and their appearance at 48 h p.i. occurred at the same time that *enhancin 1* and *2* gene transcripts were first detected in earlier studies (Bischoff & Slavicek, 1997; Popham et al., 2001). Enhancin 1 and 2 were found in polyhedra and were further localized to ODV. The amounts of Enhancin 1 and 2 isolated from polyhedra and from ODV isolated from the same number of polyhedra were approximately the same, suggesting that most, if not all, Enhancin 1 and 2 present in polyhedra were located within ODV. Enhancin 1 and 2 were found not to be components of BV. Treatment of ODV with detergents indicated that Enhancin 1 and 2 were present in ODV envelopes, and that the Enhancins may have an ionic bond with the nucleocapsid through positively charged amino acids. Immunogold labeling of polyhedron sections localized the Enhancins to ODV envelopes, either at the outside edge or within the envelope. The location of LdNPV enhancins within ODV envelopes (Slavicek and Popham, 2005) would be consistent with the hypothesis of a role in binding to midgut cells. A general PM degradative function of GV enhancin is consistent with their location and amount within the GV granule. However, this does not preclude a duel function of binding to and facilitating entry into midgut cells. Such a duel role for GV enhancin has never been tested within a homologous context. As noted earlier, the presence of LdMNPV enhancins within ODV envelopes provides a position for the proteins to interact directly with midgut cells as well as on the PM as ODV traverses this host barrier.

To determine if the LdMNPV enhancin's sole function was degradation of the PM, a fluorescent brightener was used to eliminate the PM, and the impact on the potency of recombinant virus lacking both *enhancin* genes was determined (Hoover et al., 2010). Removal of the PM should eliminate the reduced potency of the recombinant virus lacking both *enhancin* genes compared to wild-type virus. The potency of the *enhancin* gene double

deletion virus was about 14-fold less potent compared to wild-type virus in brightener treated larvae. These results suggest that the LdNPV *enhancin* genes have a function that impacts viral potency that does not involve degradation of the PM. The findings that the two enhancin proteins can partially compensate for the lack of the other (Popham et. al., 2001), their location in the ODV envelope (Slavicek & Popham, 2005), and the reduced potency of *enhancin* gene deletion viruses in the absence of the PM support the hypothesis that one or both of the enhancins are involved in a function that impacts viral potency, such as binding to midgut epithelial cells in addition to disruption of the PM. Further studies are needed to address this possibility.

4.4 Alpha-helix transmembrane domain analysis of NPV and GV enhancins

Analyses of LdMNPV Enhancin 1 and 2 for the presence of transmembrane alpha-helices by the DAS method (http://www.sbc.su.se/_miklos/DAS/), the PRED-TMR Algorithm (http://o2.db.uoa.gr/PRED-TMR), TMHMM (www .cbs.dtu.dk/services/TMHMM/), and SOSUI (http://sosui.proteome.bio.tuat.ac.jp/sosuiframe0.html) all predict the presence of a transmembrane alpha-helix in LdMNPV Enhancin 1 in the amino acid region from about positions 738 to 761 and in Enhancin 2 in the amino acid region from about positions 747 to 771 (Table 4) (Slavicek & Popham, 2005). The TMHMM method further predicts that amino acids of Enhancin 1 from positions 1 to 737 and of Enhancin 2 from positions 1 to 746 are located on the outside of the membrane structure, residues 738 to 760 (Enhancin 1) and 747 to 769 (Enhancin 2) span the membrane, and amino acids 761 to 782 (Enhancin 1) and 770 to 788 (Enhancin 2) are located on the inside of the membrane structure (i.e., next to the nucleocapsid). These predictions suggest that the carboxyl-terminal regions of Enhancin 1 and 2 are anchored within the ODV envelope, and that the majority of the proteins extend beyond the envelope surface. An interesting correlation is that many of the gold particles were found at the outside edge of ODV envelopes after immunogold labeling, which is consistent with the Enhancin 1 and 2 epitopes (regions 279 to 298 and 506 to 525, respectively) being located on the outside of ODV envelopes. If this orientation were present, the LdMNPV enhancins would be positioned to interact with the peritrophic membrane within the host midgut. It is interesting that the regions from positions 761 to 782 of Enhancin 1 and 770 to 788 of Enhancin 2 each contain six basic amino acids, which may bind the enhancins to the nucleocapsids (Table 5).

Analysis of the NPV enhancins with TMHMM, PRED-TMR2, DAS and SOSUI transmembrane protein structure prediction programs predicted the presence of alpha-helix transmembrane domains near the carboxyl terminus in all NPV enhancins (Table 4). In contrast, none of the GV enhancins were predicted to contain transmembrane protein structures, and the programs classified them as soluble proteins. The lack of GV enhancin transmembrane domains and classification of GV enhancins as soluble proteins are consistent with their localization as components of granules. All of the NPV enhancins contain from 2 to 9 basic amino acid residues between the end of the transmembrane region and the carboxyl terminus (Table 5). The CfNPV enhancin contained the least (2) and AgipMNPV contained the most (9) basic residues. All of the basic residues in this region in MacoNPV-A, HearMNPV, and Maco-B were lysine residues and the other NPV enhancins contained arginine and lysine residues, lysine and histidine residues, or all three (Table 5).

	Amino Acids Constituting the Predicted Alpha-Helix Transmembrane Domain by the Respective Prediction Program			
Viral Enhancin	TMHMM	PRED-TMR2	DAS	SOSUI
LdMNPV-VEF-1	738-760	744-761	738-761	738-760
LyxyMNPV-VEF-1	740-762	746-763	739-763	739-761
EupsNPV	761-783	763-779	761-782	762-784
LyxyMNPV-VEF-2	747-769	750-768	748-771	749-771
LdMNPV-VEF-2	747-769	750-768	748-771	749-771
CfMNPV	719-741	720-740	718-741	719-741
AgseNPV-VEF-1	819-841	822-841	816-843	817-839
MacoNPV-B	806-828	805-824	803-823	803-825
HearMNPV	806-828	805-824	803-827	803-825
AgseNPV-VEF-2	827-849	825-844	822-849	827-849
AgipMNPV	827-849	827-846	823-852	828-850
MacoNPV-A	804-826	804-823	803-826	803-825
XecnGV-VEF-2	---	---	---	---
HearGV-VEF-2	---	---	---	---
HearGV-VEF-3	---	---	---	---
PsunGV-VEF-3	---	---	---	---
TnGV	---	---	---	---
CfGV	---	---	---	---
XecnGV-VEF-3	---	---	---	---
XecnGV-VEF-4	---	---	---	---
HearGV-VEF-4	---	---	---	---
PsunGV-VEF-4	---	---	---	---
XecnGV-VEF-1	---	---	---	---
HearGV-VEF-1	---	---	---	---
PsunGV-VEF-1	---	---	---	---
AgseGV	---	---	---	---

Table 4. Analysis of NPV and GV Enhancins for Transmembrane Alpha-helices and Carboxyl Terminal Basic Amino Acids

Viral Enhancin	Amino Acid End of Transmembrane Domain	Downstream Sequence[a]
LdMNPV-VEF-1	761	VNRRGRQSPKAAERAPPLQRV
LyxyMNPV-VEF-1	763	VNQRGRQNPKAAEHAPLQHS
EupsNPV	779	KLVYSKTTNITESTPLMLDRQQT
LyxyMNPV-VEF-2	768	IATIARRAKRDDARPPSSIKA
LdMNPV-VEF-2	768	TIARRAKRDDARPPSVIKA
CfMNPV	740	KNMATPNTSHNLAPNIS
AgseNPV-VEF-1	841	LKVTRAQETAPLTPIPAISAPIASAPTQTRRRRK IIE
MacoNPV-B	824	PNAIETIIKEKPKTNIKSIK
HearMNPV	824	IKIASPSKKQVITKEKPKPVIKSIK
AgseNPV-VEF-2	844	VKFLVGANKCAIAETIQAPPPPGKTITTRRPTR VTPITTA
AgipMNPV	846	IKLIVSPNCVVYQTSPSLPPPPAASARTRTARN VTSRRPTRGVINRSPTPTR
MacoNPV-A	823	SPSKKQVITKEKPKPVIKSIK

a. Arginine residues are highlighted in turquoise, histidine residues in green, and lysine residues in yellow.

Table 5. Location of Basic Amino Acids Downstream of the Alpha-helix Transmembrane Domain

4.5 Applications of baculovirus enhancins for insect pest control

Researchers have been working to develop means of using baculovirus *enhancin* genes to increase the efficacy of insect control efforts. The impact on potency of AcMNPV by TnGV enhancin was indirectly investigated by combining cell culture cells infected with a recombinant virus containing the TnGV *enhancin* gene. The potency of AcMNPV and SeNPV were 21-fold and 10-fold greater, respectively when the cell culture cells infected with the enhancin expressing AcNPV recombinant were present (Hayakawa et al. 2000). More recently, a recombinant AcMNPV was generated that expressed the TnGV *enhancin* gene. Polyhedra from the enhancin expressing virus were found to be approximately 2-fold more potent compared to wild-type AcMNPV polyhedra (Del Rincon-Castro & Ibarra, 2005). Insertion of the MacoA *enhancin* gene into AcMNPV increased the potency of the recombinant virus approximately 4-fold (Qianjun et al., 2003). The TnGV *enhancin* gene was also placed into the genome of tobacco and found to impact viral potency. Larvae feeding on diet containing dried tobacco leaves expressing TnGV enhancin exhibited a 10-fold enhancement of AcMNPV infection (Hayakawa et al., 2000). TnGV enhancin was found to increase the toxicity of *B. thuringiensis* (Berliner) in larval bioassays with *T. ni*, *H. zea*, *H. virescens*, *S. exigua*, *P. includes*, and *A. gemmatalis* (Granados et al., 2001).

5. Conclusions

The location of NPV enhancins within ODV envelopes is likely a conserved characteristic of all NPV enhancins, in contrast to GV enhancins being located within granules. This difference suggests that the NPVs and GVs utilize distinct approaches to degradation of the PM. Results to date indicate that GVs release a large amount of enhancin into the larval midgut, which then degrades the PM in a non-targeted random manner. In contrast, the NPVs may utilize enhancins located in ODV envelopes to "tunnel" through the PM to gain access to larval midgut cells. The presence of transmembrane domains in the carboxyl terminus of all NPV enhancins known to date suggests that the localization of LdMNPV enhancins to ODV envelopes is a conserved feature of NPV enhancins. Whether this is a conserved characteristic will require analyses of the locations of enhancins in additional NPVs. The finding that deletion of LdMNPV enhancins negatively impacts viral potency in the absence of the PM supports the hypothesis that the LdMNPV enhancins have a function that impacts viral potency that is distinct from degradation of the PM. This could support earlier hypothesis suggesting that GV enhancins increase NPV potency in heterogonous systems through ODV binding to midgut epithelial cells. The use of NPV and GV enhancins to increase the potency of NPVs lacking enhancins and impact on NPV potency when expressed in plants indicates that enhancins can be used to increase insecticidal activity of NPVs and may be useful for commercial pest control applications.

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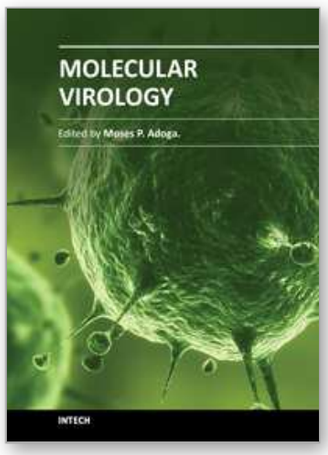

Molecular Virology

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This book covers various aspects of Molecular Virology. The first chapter discusses HIV-1 reservoirs and latency and how these twin phenomena have remained a challenge to eradication. Aspects regarding the molecular evolution of hepatitis viruses including their genetic diversities with implications for vaccine development are treated in the second chapter. Metabolic disorders that are a consequence of hepatitis C virus infection are discussed in the succeeding chapter. The following two chapters discuss influenza C virus and the applications of viral vectors in therapeutic research. Avian influenza is handled in the sixth chapter and the therapeutic potential of belladonna-200 against japanese encephalitis virus infection is discussed in the succeeding chapter. The last two chapters discuss baculoviruses and their interaction with polydnaviruses. Researchers, lecturers and students will find this book an indispensable companion.

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