Apoptosis: Its role in microbial control of insect pests

K. Narayanan

Project Directorate of Biological Control, P. B. No. 2491, H. A. Farm (PO), Bangalore 560 024, India

Very little is known concerning insect defence against virus infection. Insect haemocytes can provide cell-mediated immunity to bacterial pathogens through phagocytes and encapsulation. Neither cell mediated nor humoral immunity has been demonstrated against virus infection in insects. 'Apoptosis' is a distinctive type of programmed cell death, a phenomenon evolved as a primitive viral defence in certain vertebrate animals and invertebrates. In these system lacking humoral immunity, antiviral defence mechanism is gaining importance in cellular defence against viral infection. However, insect baculoviruses like nuclear polyhedrosis virus, granulosis virus and other DNA viruses of insects evolved methods apparently to bypass this defence phenomenon of apoptosis by directly blocking this response with the possession of *p35* gene. The future possibility of blocking the apoptosis for increasing the virulence and host range of certain baculoviruses has been presented. It is very important for assessing the potential risk of genetically improved baculoviruses. Further understanding the mechanisms for the development of robust cells for *in vitro* multiplication of insect viruses have also been discussed in this paper.

INSECTS are among the earliest and most successful group of animals that exist in a myriad of environment where the potential for infection by various types of microorganisms and parasites is great. The defence mechanism is broadly classified into two groups. The first one is non-specific immunity which consists of structural and passive barriers like cuticle, gut physiochemical properties and peritrophic membrane. The second is specific immune system involving cellular and humoral immunity.

Much information is available on insect defence against fungal and bacterial infection by way of insect haemocyte providing cell-mediated defence to fungal and bacterial pathogen through phagocytes and encapsulation and synthesis and release of several anti-bacterial (immune) proteins like cecropins, attacins, diptericins, defensins and enzymes like phenol oxidases and lysozymes, etc.[1] Very little is known concerning insect defence against virus infection. In fact insects lack lymphocytes, the major source of vertebrate immunity to virus infection, although they do have haemocytes[2,3] through which infected cells are encapsulated and subsequently cleared[4], but a role in response to viral infection has not been demonstrated. However as a part of survival strategy, insects have evolved methods, in the absence of antibody-mediated immune response; effective defence mechanism to resist virus infection. One such mechanism is through apoptosis – a programmed cell death. Similarly some DNA containing viruses have means of circumventing apoptosis too.

In order to appreciate the apoptosis and its role in microbial control of insect pests by the conventional Agricultural Entomologists and Applied Insect Virologists, some preliminary information on different aspects of apoptosis, viz., definition, its morphological and biochemical features, along with some of the genes/gene products that are involved in the regulation of apoptosis both in vertebrates and in invertebrates has been presented in general in this review article. Further, its mechanism of induction and inhibition by way of carrying apoptotic and anti-apoptotic genes by different groups of insect viruses namely, baculoviruses (consisting of nuclear polyhedrosis virus and granulosis virus), iridovirus and other DNA viruses like ascovirus, polydnavirus and non-occluded viruses has been presented in particular. All these insect DNA viruses have evolved methods to by-pass this defence mechanism, i.e. either they have the ability to block cellular apoptosis as part of their invasion strategy or evolved unusual strategy to circumvent apoptosis. Further, future possibility of identifying genes which are responsible for induction and inhibition of apoptosis by various insect viruses has also been highlighted. For more detailed information on the mechanism of apoptosis induced by other DNA containing vertebrate and mammalian viruses, the readers may refer references 5–11.

Definition

Apoptosis is a distinct type of programmed cell death, a phenomenon evolved as a primitive viral defence in certain vertebrate animals and invertebrates. In these systems lacking humoral immunity, to function as an an-

tiviral defence mechanism is gaining importance in cellular defence against viral infection[12,13]. Apoptosis may be defined as 'a process where the cells die in a controlled manner in response to specific stimuli, apparently following an intrinsic programme'. The word apoptosis was coined by Kerr and Searle[14] which in Greek means 'falling off'. It is an active process of cellular self-destruction with distinctive morphological and biochemial features. It serves an essential function for the multicellular organism, for embryogenesis, differentiation, metamorphosis and for tissue homeostasis by way of providing counter balance to mitosis in normal adult tissues.

Morphological and biochemical changes associated with apoptosis

Two common forms of cell death have been described in vertebrate tissues. One is necrosis and the other apoptosis. Necrosis refers to the morphology most often seen when cells die from severe and sudden injury from an external agent such as ischemia, restrained hyperthermia or physical or chemical trauma. It is a non-specific form of cell death. Further, it is characterized by cell swelling, loss of plasma membrane integrity, and finally spillage of the cell contents upon rupture of the cell, resulting in an inflammatory response[7].

Whereas in the case of apoptosis, cell death appears to be programmed by genetically-controlled pathways. It is characterized by controlled auto-digestion of cells. Cells appear to initiate their own apoptotic death through the activation of endogenous proteases. This results in cytoskeletal disruption, with distinct morphological changes like condensation of chromatin in the nuclear periphery, nuclear collapse, cell shrinkage, membrane blebbing, lack of cell to cell contacts and formation of apoptotic bodies (nuclear and cytoplasmic fragments enclosed in plasma membrane). Apoptosis may either be taken up by adjacent cells in epithelia or they may be sloughed off. Either the resident cells phagocytose the apoptotic bodies or macrophages sometime play a role in the removal of apoptotic bodies. This type of cell death occurs in individual cells in an asynchronous fashion which also differs from necrosis. Unlike necrosis, the absence of inflammatory reaction in the case of apoptosis is the characteristic feature of the apoptosis. The DNA fragmentation is in multiples of 180–200 bp of characteristic DNA 'ladder' as opposed to the random DNA fragmentation seen in necrosis. Further, apoptosis is an individual and active type of cell death that is characterized by nuclear fragmentation and cellular breakdown into apoptotic vesicles. Unlike necrosis, there is no release of cellular contents into the interstices and consequently no inflammation surrounding the dead cells. In this type of cellular self-destruction, it is usually initiated by physiological stimuli but pathological infections can also trigger apoptosis[7]. In this article, we review mainly the induction and inhibition of apoptosis due to insect virus infection. For more details on the different intra and extra-cellular signals in insects like steroid-moulting hormone ecdysone, which may either promote or suppress the initiation of cell death, along with the signal pathway model, the interested readers may refer references 15–18.

Range of viruses carrying apoptotic genes

A variety of DNA containing viruses have molecular strategies for preventing programmed cell death, specifically apoptosis. Members of atleast three distinct virus families, Herpesviridae, Adenoviridae and Baculoviridae are known to carry genes that function to block cellular apoptosis. Many viruses have evolved mechanisms to inhibit programmed cell death and thereby sustain host cell viability to facilitate virus multiplication. Viral inhibition of programmed cell death can also contribute to the establishment of a latent infection through cellular transformation by expression of viral gene products. Adenoviruses, for example, induce programmed cell death after infection of cells by expression of the EIA viral proteins which stimulate cellular DNA synthesis and thereby increase viral replication. Expression of other viral genes, for instance, the adenovirus *eib* genes results in the inhibition of apoptosis. This can either enhance virus production, as in the case of adenovirus, or establish a persistent infection as in the case of Epstein-Barr virus[6]. Recently Antoni *et al.*[19] have shown that inhibition of apoptosis in human immuno deficiency virus-infected cells enhances virus protection and facilitates persistence. It has been suggested that the ability to block cellular apoptosis is a part of the invasion strategy of most DNA containing viruses of not only thoroughly studied viruses like insect baculoviruses, but also other DNA containing insect viruses. Further, it is also reported in the currently unclassified and barely studied insect virus group known as insect polydnavirus and ascovirus and even non-occluded baculovirus known as Hz-1. They have all evolved an unusual strategy for cirvumventing apoptosis by using the response as part of their replication pathway to cellular apoptosis which will be discussed later.

Apoptotic and anti-apoptotic genes

Apoptosis is a genetically-controlled process. Many virus genomes encode gene products that modulate apoptosis, either positively or negatively, and induction of apoptosis often contributes directly to the cytopathogenic effects of the viruses, on the other hand, may pre-

vent premature death of the infected cells, thereby facilitating viral replication, spread or persistence. The following are some of the genes/gene products that are involved in the regulation (i.e. induction and inhibition) of apoptosis both in vertebrates and invertebrates.

Apoptotic genes

Vertebrates

i. The *c-myc* gene which encodes nuclear phosphoprotein has been shown to induce apoptosis[20].
ii. Cysteine protease interleukin-1B-converting enzyme (ICE)[21].
iii. Adenovirus induces apoptosis by expression of the EIA viral protein through *eia* gene[22].
iv. The tumour suppressor gene (*p53*).

The function of *p53* is to arrest cells in the G1 phase of cell cycle following DNA damage. This allows the cell lines to repair the damage before DNA synthesis occurs. If the damage cannot be repaired, then apoptosis is triggered. If *p53* fails to perform its duties, perhaps due to mutants, then cells with genetic damage can re-enter the cell cycle possibly giving rise to cancer[6].

Invertebrates

Nematodes

A protein required for programmed cell death in the nematode, *Caenorhabditis elegans* is cell death protease (*ced-3*)[6].

Insects

Though genes which are responsible for inducing apoptosis were not reported in insect baculoviruses, induction of apoptosis by deficient mutant (vAcAnh) of AcMNPV (*Autografa californica* multiple embedded nuclear polyhedrosis virus) which is a cell type specific, i.e. cell death occurring in some insect cells, Sf-21 (cell line derived from *Spodoptera frugiperda*) and Bm N4 (cell line derived from *Bombyx mori*) and not in others, Tn-368 cells (derived from *Trichoplusia ni*) has been reported by Clem *et al.*[23]. Recently Prikhod'Ko and Miller[24] have demonstrated that transient expression of the AcMNPV '*ie-i*' gene induced apoptosis in Sf-21 cells but not in Tn-368 cells just like *p35* deficient mutant (vAcAnh) of AcMNPV virus. Palli *et al.*[25] have found that wild AcMNPV induces apoptosis and failed to replicate in *Choristoneura fumiferana* mid-gut cell line (Cf-203); whereas the same wild AcMNPV replicate and reproduce occlusion bodies in an ovarian cell line (FPMI Cf-70) of the same insect. Apoptosis was postulated as the main barrier to replicate *Autographa californica* nuclear polyhedrosis virus (AcMNPV) in *Spodoptera littoralis* (Sl-2) cell line by Chejenovsky and Gershberg[26]. But recently they have also suggested that apoptosis is not the only impediment to AcNPV replicaion in the non-permissive *Spodoptera littoralis* cells and larvae[27].

Anti-apoptotic genes

Vertebrates

Human proto-oncogene (*bcl-2*) (ref. 6).

Invertebrates

Nematodes

Caenorhabditis elegans bcl-2 analogue (*ced-9*) (ref. 6).

Insect viruses

Autografa californica multiple embedded nuclear polyhedrosis virus (AcMNPV), carries apoptosis gene *p35* in addition to '*iap*' gene, that works in conjunction with '*iap*' to inhibit apoptosis in a wider range of insects. This could explain the relatively broad host range exhibited by AcMNPV compared to other baculoviruses with a narrower host range. Whereas in the case of *Orgyia pseudotsugata* NPV (though it is closely related to AcMNPV), since it lacks *p35* gene, it has a narrower host range unlike that of AcMNPV (ref. 28). However, very recently an *iap* homologue (*Op-iap* gene) that functions to inhibit apoptosis has been identified in this case[29]. Nucleotide sequence analysis of the *B. mori* nuclear polyhedrosis virus (BmNPV) genome revealed the existence of a gene homologous to the *p35* gene of *A. californica* NPV (AcNPV), which has been shown to prevent virus-induced apoptosis[30]. In the case of codling moth of apple, *Cydia pomonella* granulosis virus, '*iap*' gene (inhibitor of apoptosis) was isolated (*Cp-iap*)[31]. Occurrence of a homologue of *Cp-iap* and *Ac-iap* in the genome of an insect iridovirus[32] suggests that these DNA containing viruses also have means of circumventing apoptosis.

Mechanism

The death of an individual cell is an integral and continuing part of the normal physiology. It is also a major form of a defence, as sometimes the only way for the immune system to eradicate the pathogens is to sacrifice the infected cell. When the timing of the cell death is

inappropriate, however, havoc may ensue. So, understanding the mechanisms that regulate cell death is just as important as understanding those responsible for the actual killing. The nematode, *Caenorhabditis elegans* has been used with great success to identify the basic components of the machinery underlying apoptosis[33]. Indeed all the three key cell death genes that have been identified in *C. elegans, ced-3, ced-4* which control general apoptotic programme and *ced-9* which negatively regulates the apoptotic programme have mammalian homologues[34–36]. A newly-identified protein appears to act as a regulator of the principal pathways by which immune mechanism causes cell death. In the case of death induction, latent precursors of caspases (a family of cysteine proteases) are activated early in the process of programmed cell death and central to its development[21].

The molecular mechanisms by which baculoviruses induce apoptosis are unknown[37]. Similarly the molecular mechanism by which the *p35* gene product block apoptosis has not yet been elucidated when this phenomenon was noticed during 1991 by Clem *et al.*[23]. It is an early viral protein[38] and its early expression during viral infection is required to block apoptosis. Predicted amino acid sequence of *p35* has no obvious homology to other known protein sequences[23]. The fact that expression of *p35* alone can prevent apoptosis indicates that its function in blocking apoptosis is not mediated by other viral proteins. It localizes predominantly to the cytosol, and only a minor portion of its membrane bound[39]. This may suggest that the mechanism by which *p35* blocks apoptosis is different from that of *bcl-2* which is membrane bound[40]. Considering the *p35* effectiveness in blocking apoptosis in diverse organisms, it was later demonstrated by Bertin *et al.*[41] that the *p35* gene product (P35) is a potent or competitive inhibitor of CED3/ICE related cysteine proteases (caspases) and thus preventing caspase-induced apoptosis[42,43].

Evolution

The evolutionary basis of the origin of cellular apoptosis is an intriguing question. It has been proposed that apoptosis evolved in multicellular organisms as a defence against virus infection wherein the sacrifice of a small number of cells would be beneficial to the whole organism. Expression of the mammalian *bcl-2* gene during wild type baculovirus (*p35*) infection can further prolong the survival of the infected cells and prevent DNA degradation. Further that the *p35* inhibiting the programmed cell death in a diverse range of organisms such as insects, including *Drosophila*[44], nematodes[45] and mammals[46] suggests that the mechanism regulating apoptosis might have been conserved during evolution.

History of apoptosis in insect viruses

Baculovirus (AcMNPV)-induced cellular apoptosis was realized when people started working with a mutant AcMNPV known as 'Annihilator', vAcAnh, isolated by Roland Russnak of the University of British Columbia, when the mutant was unable to make occluded viruses but did contain polyhedrin gene. This was demonstrated by way of infecting the Sf-21 cell line with vAcAnh, which, however results in premature 'lysis' beginning approximately 12 hours after post infection exhibiting characteristics typical of apoptosis including active blebbing and formation of apoptotic bodies and fragmented DNA. This was considered the first formal demonstration that apoptosis occurs in invertebrates as well as vertebrates, indicating an evolutionary origin for apoptosis (cited by Clem and Miller)[28].

Specificity of apoptosis

Cellular apoptosis may be induced by a variety of different extracellular and intracellular stimuli and apoptosis during viral infection is a general response of insect cells to virus infection, which can differ depending upon the cell types. Though apoptosis was first observed in Sf-21 cells, the Bmn4 cell line also undergoes apoptosis when infected with vAcAnh, whereas *p35* mutant of AcMNPV cannot induce apoptosis in Tn-368 cells[23]. Recently Palli *et al.*[25] have found similar cell type-dependent effects. They found that AcMNPV replicates and produces occlusion bodies in FPMI-Cf-70, an ovarian cell line of spruce budworm, *Choristoneura fumiferana*; whereas it induced apoptosis and failed to replicate in Cf-203, a mid-gut cell line of the same insect. These observations suggest that apoptosis may also play a role in determining the host range of baculovirus. Thus apoptosis induced by *p35*-deficient mutant is cell type specific cell death occurring in some insect cells but not in others.

Induction of apoptosis

The RNA synthesis inhibitor actinomycin D has been shown to induce apoptosis in some mammalian cell types. Recently it has been found that treatment with actinomycin D alone induces apoptosis in Sf-21 cells, resulting in both apoptotic morphology and chromatin degradation[31]. It is not known what baculovirus gene is responsible for apoptosis. But it is noteworthy that in adenovirus infections, apoptosis is induced by the E1A which interacts with (or) controls the activity of cellular proteins involved in cell cycle regulation. On the other hand, the large nuclear replicating DNA-containing viruses may inhibit apoptosis, turning off host DNA and

RNA synthesis and turning on viral DNA and RNA synthesis while blocking programmed cell death at a more distant point in the downstream pathway[28].

Apoptosis in insects by insect viruses other than occluded baculovirus

Ascovirus

Ascoviruses are a new group of viruses that cause a chronic but a fatal disease in lepidopteran larvae. The virions produced by these viruses are large (400 × 130 nm), reniform to bacilliform in shape, enveloped, and contain a genome of linear double standard DNA which, depending on the isolate, varies in size from 145 to 170 kb. The primary feature of the disease caused by ascovirus is a marked decrease in the rate of larval growth and development which begins shortly after infection. Most infected larvae fail to gain any significant amount of weight or progress substantially in development, appearing stunted in comparison to healthy cohorts. Infected larvae can remain in this arrested state of development for several weeks, but eventually die of the disease[47].

As the disease advances, the host cell divided into membrane bound vesicles (or 'sacs', containing large number of virions), formed by cleavage of infected host cells, accumulate in the haemolymph, imparting it an opaque white colour. The process of vesicle formation and the nature of vesicles themselves strongly resemble apoptosis and apoptotic bodies. These viruses are vectored by parasitoid wasps during oviposition, and the vesicle possibly represents one infectious form of the virus. Thus ascovirus may have developed an unusual strategy for circumventing apoptosis by using a part of their replication pathway. Though work on ascovirus has been reported abroad[47], no work has been done in India except the casual report on the occurrence of ascovirus in *Helicoverpa armigera* and *Spodoptera litura* by Narayanan[48].

Polydna virus

Certain female parasitic wasps in the families of Ichneumonidae and Braconidae carry the particles containing double stranded circular, multiple DNA viruses called ichnovirus and bracovirus. These viruses are infected through the cuticle during the oviposition of parasitoids. These are essential for the survival of their progeny in their habitual host. In the absence of these virus particles, the parasitoid egg is recognized as foreign and encapsulated by host blood cells, i.e. insect haemocytes, especially by granulocytes, whereas in the presence of virus the parasitoid is not encapsulated[49]. Recently, Strand and Pech[50], while studying the mechanism underlying the immuno suppression of *Pseudoplusia includens* (i.e., host) for the parasitization of *Microplitis demolitor*, found that MdPDV induced apoptosis in granular cells with characteristic condensation of chromatin, cell surface blebbing and fragmentation of DNA into a 200 bp ladder. Though most of the baculoviruses promote their own survival by suppressing apoptosis of host cells, the MdPDV promote their own survival by inducing apoptosis of host immune cells which would otherwise kill the developing *M. demolitor* egg.

In general, larvae of lepidopteran insects become increasingly resistant to baculovirus infection as they age. Such developmental resistance has been reported in many species of lepidopteran larvae infected with baculoviruses[51] including *H. armigera* for its own baculovirus[52]. Though much work has been done on the polydna virus (PDV), and its role in understanding the abrogation of host insect defence mechanism[53,54], no work has been done in India. Generally polydna viruses have been reported on ichneumonid and braconid parasitoids of hymenopteran insects. However, the susceptibility of late 5th instar of *H. armigera* (which is immune) to HaNPV immediately after parasitization by a tachinid parasitoid *Eucelatoria* sp. breaking the maturation immunity[55] suggest the possibility of the presence of some unknown factors/particles or virus similar to polydna of hymenopteran insects. They might have altered the immune system by way of inducing apoptosis in the key immune cells like granulocytes. They mediate encapsulation process in the beginning, leaving many other putative replacement cells, (which are not infected owing to the absence of free polydna virus 24–36 h post parasitization), as observed by Strand[56], for HaNPV infection by way of inhibiting apoptosis. The immune suppression of *Drosophila melanogaster* for the parasitization of *Leptospilina heterotoma*, a cynipid wasp reported by Rizki and Rizki[57] suggest that other than braconids and ichneumonids, cynipids can also alter the host developmental immune response.

A tachinid parasite *Eucelatoria bryani*, which was introduced from USA, during 1980 (ref. 58) for the control of *H. armigera*, was discontinued in India because of its poor performance by this parasite alone (since only one and rarely two maggots comes from a single parasitized host larva). The study on the interaction between HaNPV[55] and *Eucelatoria* revealed susceptibility of the recalcitrant grown up *H. armigera* larvae to NPV infection. The above study paves the way for its better utilization in future in the light of the following considerations: (i) Larvae of many dipteran parasitoids especially tachinids maintain contact with outside air by attaching their posterior spiracles to the host's tracheal system or a hole in the integument[59]. In many instances, the larvae of these parasitoids turn the immune response

of their host to their own advantage by building a respiratory funnel. The presence of such a respiratory funnel allows the developing parasitoid continuous access to fresh air through the host's tracheal system or hole in the host's integument. (ii) Recently, Engelhard *et al.*[60] have discovered that the tracheal system is the major conduit for baculovirus movement through infected hosts. And the finding that the larvae can be infected directly via the tracheal system has profound implications for the use of baculoviruses as pest control agents. (iii) Availability of another species of *Eucelatoria*, viz. *Eucelatoria rubentis* which has four times broader host range than *E. bryani*[61]. Further, the incorporation of polydna virus genes with immuno suppressant activity into the genome of HaSNPV in future might lower resistance in grown-up larvae of *H. armigera* and enable this pest to be controlled even in the grown up stage with a recombinant HaSNPV. The recent study by Washburn *et al.*[4] by way of allowing *C. sinorensis*, an inchneumonid parasite to oviposit into *H. zea* larvae, which is highly resistant to AcMNPV, immediately before the *H. zea* larvae were orally inoculated with AcMNPV, supports the above concept.

Non-occluded virus

The non-occluded baculoviruses (NOB) do not produce occlusion bodies at any stage in their reproductive cycle. The type species of NOB is the *Heliothis* NOB. The *Heliothis* non-occluded virus (HzNOB) was isolated from the *Heliothis zea* cell line. The Hz-1NOB is a persistent virus in tissue culture and is apparently not infectious to *H. zea* either by feeding or intrahaemocoelic inoculation. It can be transmitted to a number of lepidopteran cell lines[62].

The replication of Hz-1NOB is generally similar to other baculoviruses, except that viral assembly in the nucleus begins and ends in membrane vesicles. Virions of Hz-1NOB (enveloped nucleocapsids) enter the cytoplasm after the breakdown of the nuclear envelope but do not exit (exocytosis) through plasma membrane. This is a major difference from other baculoviruses. The virions are released only after cell lysis which occurs in a few cells. Whereas non-occluded baculovirus known as Hz-1 has evolved yet another strategy unlike that of other occluded baculovirus, or ascovirus or polydna virus, to respond to cellular apoptosis.

In a typical wild baculovirus infection, polyhedral occlusion bodies are made and are usually visible during the late phase of infection which initiates between 18 and 24 h post infection. On the other hand, AcMNPV mutant vAcAnh induces apoptosis around 9–12 h after infection. Whereas in the case of non-occluded Hz-1, it is able to complete its replication rapidly by about 12 h post infection, before apotosis occurs. Thus, it is able to circumvent the apoptosis efficiently. Hz-1 is a persistent baculovirus in some host cells and does not have an occluded form or an occlusion phase[62]. During the lytic phase of infection, host cell lysis begins around 12 h post infection, but sufficient progeny virus has been produced to continue the infection.

Apoptosis: Its role in microbial control of insect pests

Understanding host specificity

Baculoviruses are generally quite host specific, such as *Helicoverpa* NPV and *Orgyia pseudotsugata* NPV. Their infection is limited to a single species or a few closely-related species of insects. AcMNPV on the other hand has a broader host range than many baculoviruses both *in vitro* and *in vivo*, reportedly infecting atleast 33 species of lepidopteran larvae in 10 families as well as more than 25 different cell lines[63].

In baculoviruses, there are two different genes, *p35* and *Iap*, that are capable of overcoming apoptosis. '*Iap*' is the primary apoptosis-inhibiting gene carried by baculoviruses but some baculoviruses, such as AcMNPV, have acquired additional gene (i.e. *p35*) that works in conjunction with *Iap* to inhibit apoptosis in a wider range of insects. This may be the reason for the relatively broad host range exhibited by AcMNPV compared to other baculoviruses. On the other hand, *Orgyia pseudotsugata*, though closely related to AcMNPV baculovirus, lacks *p35* gene, thereby showing the narrower host range[28]. The impact of apoptosis in increasing the virulence and enhancing the host range of certain insect viruses has been dealt with in the following case study.

Case study: Spruce budworm, Choristoneura fumiferana NPV. Palli *et al.*[25] have reported that AcMNPV replication is blocked in *C. fumiferana* insect midgut cell line (Cf-203 cells), but restored upon pre-infection with *C. fumiferana*, multiple embedded nuclear polyhedrosis virus (CfMNPV) atleast 24 h prior to infection with AcMNPV. They also suggested that apoptosis is induced because of this replication defect, which does not occur upon infection with CfMNPV alone. Co-infection of AcMNPV with CfMNPV restores transcription of AcMNPV late genes such as *Ac-iap*, *Ac-pol*, and *Ac-p10* which are not detected in Cf-203 cells infected only with AcMNPV. Apparently, CfMNPV provides a factor(s) that overcomes this block, prevents apoptosis, and allows AcMNPV replication. Pre-inoculation of Cf-203 (which is originally not permissive to AcMNPV) at least 24 h prior to inoculation with AcMNPV provide the protection and enhancement of AcMNPV replication in

Cf-203 cells and thus CfMNPV prevents apoptosis and restores AcMNPV replication.

Production of polyhedral occlusion bodies

Clem and Miller[64] have found a reduction in the polyhedral occlusion bodies (POB) production atleast 1000-fold in *S. frugiperda* larvae infected with *p35* mutant of AcMNPV, viz., vAcAnh, which induced apoptosis in *S. frugiperda* cell line (Sf-21 cells) when compared to wild AcMNPV. Whereas occluded virus production was similar between vAcAnh and wild AcMNPV in *T. ni* larvae and cells (Tn-368) which do not allow apoptosis to occur.

Virulence

The LD_{50} value of *p35* mutant of AcMNPV was approximately 1000-folds higher than that of wild AcMNPV when tested on *S. frugiperda* larvae by budded virus infection (since Sf-21 cells undergo apoptosis when attacked by viruses lacking *p35*). The *p35* mutants and wild virus had similar LD_{50} values in *T. ni* larvae (since Tn-368 cells do not undergo apoptosis)[64].

Advantages of studying apoptosis in insect baculovirus system

The above results, i.e. the LD_{50} values of *p35* mutants of AcMNPV were higher than that of wild AcMNPV when tested in whole organism of *S. frugiperda* larvae, whereas the *p35* mutants and wild AcMNPV had similar LD_{50} values in *T. ni* larvae suggesting that death of a single infected cell could potentially protect the rest of the organism from infection. Thus insect baculoviruses provide a novel and valuable system for evaluating the role of apoptosis in an organism's defence against viral invasion, as suggested by Clem and Miller[28].

In vitro production of virus

The use of insect viruses, especially insect baculoviruses, as one of the major components in biological control of crop pests for integrated pest management appears to be gaining general acceptance as a realistic goal. At present several baculoviruses are produced commercially by the insectory method, using artificial diets with little or no sterility precautions[65]. The recent advances in laboratory culturing of insect cells might offer a satisfactory alternative method for large-scale production of insect viruses[65]. The advantages of *in vitro* application of insect viruses rather than *in vivo* are several folds, viz. being free from other viral, microbial contamination, comparatively easy; since insect cell can be maintained at room temperature 26–28°C and use of pH indicator and trypsin treatments not being necessary. There is no requirement of CO_2, generation time is generally short (16–18 h), and plaque assaying is easy. Further, insects can be cultured/maintained under serum-free synthetic media with associated advantage of low cost, less batch variation in quality, freedom from contaminating micro-organism, etc. which not only helps *in vitro* mass production of insect viruses but also in the downstream processing of protein products[66]. Further, insect cells can be cloned and stored and provide stable and uniform production of insect viruses. In fact the development of baculovirus as expression vector for foreign gene expression and production of array of proteins of agriculture/veterinary/medical/pharmaceutical importance renewed the interest of insect cell culture. In recent years, large scale animal and insect cell cultures are used by the biotechnology and pharmaceutical industry for the production of diagnostic and therapeutic proteins[67–70]. As a result, there has been considerable interest in optimization of production schemes[71].

The main focus has been on developing strategies for optimizing culture conditions for cell growth. The manipulation of culture medium, optimization of growth methods[72], agitation and aeration levels[73], pH and bioreactor design for growth of shear sensitivity of mammalian and insect cell[74] and bubble-column design for growth of fragile insect cells[75] have all led to improvements in cell culture conditions. However, little interest has been shown in controlling the level of cell death, largely because it was thought that cells die by the passive process of necrosis and little could be done to alter this as suggested by Cotter and Ai-Rubeai.

Cell death in cultures invariably takes place by the process of apoptosis, atleast in response to the low levels of environmental stress that cells are likely to experience in culture vessels. With our recent understanding of the genetics and cell biology of apoptosis, there is tremendous scope for the manipulation of cells by transfecting anti-apoptotic genes like *bcl-2* or supplementing the culture medium with appropriate survival factors (cytokines including some of the interleukins and colony-stimulating factors) or anti-apoptotic compounds such as zinc-ions, aurintricarboxylic acid (ATA) and anti-oxidants of various sorts, it is possible to enhance the robustness and survival of cells in culture[76].

Conclusion

The incorporation of polydnavirus genes through parasitoids with immuno suppressant activity like prevention of recognition, encapsulation and destruction of parasitoid eggs into the genome of respective host insect vi-

ruses might lower the resistance. Further, it will also make other recalcitrant pest species to become susceptible to the same virus. Recently, Washburn *et al.*[4] have shown this by way of allowing an ichneumonid parasite *C. sinorensis* to oviposit into *H. zea* larvae, which is highly resistant to AcMNPV. Further identification of genes like '*p35*' or its homologues such as *Op-iap, Cp-iap*, inhibit the apoptosis and thereby enhance AcMNPV replication in such non-permissive insect hosts. And engineering a recombinant AcMNPV bearing '*p35*' or its homologues or with all others baculovirus genes that have been implicated in host-range determination like *Ld-hrf-1* (*Lymantria dispar* host range factor[77]), *p143* (a baculovirus encoded protein with homology to DNA helicase[78]) and *hcf-1* (host cell specific factor[79]) would probably be more virulent and enhance the host range of certain baculoviruses. A hybrid baculovirus derived from AcMNPV and BmMNPV was constructed by Konda and Maeda[80] which is more virulent with a broader host range. Recently Lee *et al.*[81] have shown the insecticidal activity of a recombinant baculovirus containing an antisense *c-myc* gene which has been shown to be an important regulator in the determination of various facets of a cell, namely proliferation, arrest, differentiation and apoptosis. It is evident from the above that the cellular defensive strategies in insects and insect viral offensive strategies are co-evolved. Hence, understanding of which genes play what roles in which tissues of which species is very much important. Finally, disarming insect defenses both at cellular and organismal levels will provide information necessary to control or to modify host range properties of the virus in future[82].

1. Narayanan, K., *IPM Sustain. Agri. – Ent. Appr.*, 1996, **6**, 1–5.
2. Narayanan, K. and Jayaraj, S., *Madras Agric. J.*, 1973, **60**, 640–641.
3. Narayanan, K. and Subramaniam, T. R., *Madras Agric. J.*, 1975, **62**, 449–452.
4. Washburn, J. O., Kirkpatrick, B. A. and Volkman, L. E., *Nature*, 1996, **383**, 7667.
5. Cope, F. O. and Tomei, L. D., in *Apoptosis II: The Molecular Basis of Cell Death*, Coldspring Harbor Laboratory Press, Coldspring Harbor, 1994.
6. Shen, Y. and Shenk, T. E., *Curr. Opin. Gen. Dev.*, 1995, **5**, 105–111.
7. Cohen, J. J., *Immunol. To-Day*, 1993, **14**, 126–130.
8. McConkey, D. J. and Orrenius, S., *Trend Cell Biol.*, 1994, **4**, 370–375.
9. Smyth, M. J. and Trapani, J. A., *Immunology*, 1995, **202**, 202–206.
10. Horvitz, H. R., Shaham, S. and Hengartner, M. O., *Cold Spring Harbor Symp. Quant. Biol.*, 1994, **59**, 377–385.
11. Jacobson, M. D., Weil, M. and Raff, M. C., *Cell*, 1997, **88**, 347–354.
12. Clouston, W. M., and Kerr, J. F. R., *Med. Hypotheses*, 1985, **18**, 399.
13. Razvi, E. S. and Welsh, R. M., *Adv. Virus Res.*, 1995, **45**, 1–60.
14. Kerr, J. F. R. and Searle, J., *J. Pathol.*, 1972, **107**, 41–44.
15. Truman, J. W. and Schwartz, L. M., *Neuroscience*, 1984, **4**, 274–280.
16. Truman, J. W., Thorn, R. S. and Robinow, S., *Neurobiology*, 1992, **23**, 1295–1311.
17. Kimura, K. and Truman, J. W., *Neuroscience*, 1990, **10**, 403–411.
18. White, K. and Steller, H., *Trend Cell Biol.*, 1995, **5**, 74–77.
19. Antoni, B. A., Sabbatini, P., Rabson, A. B. and White, E., *J. Virol.*, 1995, **69**, 2384–2392.
20. Harrington, E. A., Bennet, M. R., Fanidi, A. and Evan, G. I., *EMBO J.*, 1994, 3286–3295.
21. Kumar, S., *TIBS*, 1995, **20**, 198–202.
22. Rao, L., Debbas, M., Sabbatini, P., Hockenbery, D., Korsmeyer, S. and White, E., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 7742–7746.
23. Clem, R. J., Fechheimer, M. and Miller, L. K., *Science*, 1991, **254**, 1388–1390.
24. Prikhod'Ko, E. A. and Miller, L. K., *J. Virol.*, 1996, **70**, 7116–7124.
25. Palli, S. R., Caputo, G. F., Sohi, S. S., Brownwright, A. J., Ladd, T. R., Cook, B. J., Primavera, M., Arif, B. M. and Retnakaran, A., *Virology*, 1996, **222**, 201–213.
26. Chejenovsky, N. and Gershburg, E., *Virology*, 1995, **209**, 519–525.
27. Gershburg, E., Rivkin, H. and Chejanovsky, N., *J. Virol.*, 1997, **71**, 7593–7599.
28. Clem, R. J. and Miller, L. K., in *Apoptosis II: The Molecular Basis of Apoptosis in Disease*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1994, pp. 89–110.
29. Birnbaum, M. J., Clem, R. J. and Miller, L. K., *J. Virol.*, 1994, **68**, 2521–2528.
30. Kamita, S. G., Majima, K. and Maeda, S., *J. Virol.*, 1993, **67**, 455–463.
31. Crook, N. E., Clem, R. J. and Miller, L. K., *J. Virol.*, 1993, **67**, 2168–2174.
32. Handermann, M., Schnitzler, P., Rosen-Wolff, A., Raab, K., Sonntag, K. C. and Darai, G., *Virus Genes*, 1992, **6**, 19.
33. Hengartner, M. O. and Horvitz, H. R., *Curr. Opin. Gen. Dev.*, 1994, **4**, 581–586.
34. Xue, D., Shaham, S. and Horvitz, H. R., *Genes Dev.*, 1996, **10**, 1073–1083.
35. Zou, H., Henzel, W. J., Liu, X., Lutschg, A. and Wang, X., *Cell*, 1997, **90**, 405–413.
36. Hengartner, M. O. and Horvitz, H. R., *Cell*, 1994, **76**, 665–676.
37. LaCount, D. J. and Friesen, P. D., *J. Virol.*, 1997, **71**, 1530–1537.
38. Dickson, J. A. and Friesen, P. D., *J. Virol.*, 1991, **65**, 4006–4016.
39. Hershberger, P. A., LaCount, D. J. and Friesen, P. D., *J. Virol.*, 1994, **68**, 3467–3477.
40. Akao, Y., Otsuki, Y., Kataoka, S., Ito, Y. and Tsujimoto, Y., *Cancer Res.*, 1994, **54**, 2468–2471.
41. Bertin, J., Mendrysa, S. M., Lacount, D. J., Gaur, S., Krebs, J. F., Armstrong, R. C., Tomaselli, K. J. and Friesen, P. D., *J. Virol.*, 1996, **70**, 6251–6259.
42. Xue, D. and Horvitz, H. R., *Nature*, 1995, **377**, 248–351.
43. Bump, N. J., Hackett, M., Hugunin, M., Seshagiri, S., Brady, K., Chen, P., Fertenz, C., Franklin, S., Ghayur, T., Li, P., Mankovich, L. J., Shi, L., Greenberg, A. H., Miller, L. K. and Wong, W. W., *Science*, 1995, **269**, 1885–1888.
44. Hay, B. A., Wolff, T. and Rubin, G. N., *Development*, 1994, **120**, 2121–2129.
45. Sugimoto, A., Friesen, P. D. and Rothman, J. H., *EMBO J.*, 1994, **13**, 2023–2026.
46. Rabizadeh, S., LaCount, D. J., Friesen, P. D. and Bredesen, D. E., *J. Neurochem.*, 1993, **61**, 2318–2321.
47. Federici, B. A., Vlak, J. M. and Hamm, J. J., *J. Gen. Virol.*, 1990, **71**, 1661–1668.

48. Narayanan, K., in Annual Report of the All India Co-ordinated Research Project on Biological Control of Insect Pests (AICRP) 1992, p. 71.
49. Edson, K. M., Vinson, S. B., Stoltz, D. B. and Summers, M. D., *Science*, 1981, **211**, 582.
50. Strand, M. R. and Pech, L. L., *J. Gen. Virol.*, 1995, **76**, 283–291.
51. Engelhard, E. K. and Volkman, L. E., *Virology*, 1995, **209**, 384–389.
52. Narayanan, K., Ph D Thesis, Tamil Nadu Agricultural University, 1979, p. 204.
53. Fleming, J. G. W., *Annu. Rev. Entomol.*, 1992, **17**, 401–425.
54. Summers, M. D. and Dib-Haji, S. D., *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 29–36.
55. Narayanan, K., Paper presented at the Third Annual Workshop on Biological Control of Crop Pests and Weeds, Ludhiana, 27–30 October, 1980, p. 165.
56. Strand, M. R., *J. Gen. Virol.*, 1994, **75**, 3007–3020.
57. Rizki, R. M. and Rizki, T. M., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 8388–8392.
58. Mani, M., Nagarkatti, S. and Narayanan, K., *Entomophaga*, 1982, **27**, 399–404.
59. Askew, R. R., *Parasitic Insects*, Elsevier, New York, 1971, p. 316.
60. Engelhard, E. K., Kam-Morgan, L. N. W., Washburn, J. O. and Volkman, L. E., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 3224–3227.
61. Reitz, S. R. and Adler, P. H., *Proc. Entomol. Soc. Washington*, 1996, **98**, 625–629.
62. Wood, H. A. and Burand, J. P., *Curr. Top. Microbiol. Immunol.*, 1986, **131**, 119–133.
63. Groner, A., in *Biology of Baculoviruses* (eds Granados, R. R. and Federici, B. A.), CRC Press, USA, 1986, vol. 1, pp. 177–202.
64. Clem, R. J. and Miller, L. K., *J. Virol.*, 1993, **67**, 3730–3738,
65. Maramorosch, K., in *Biotechnology in Invertebrate Pathology and Cell Culture*. Academic Press, San Diego, 1987.
66. Goosen, M. F. A., *Curr. Opin. Biotechnol.*, 1992, **3**, 99–104.
67. Lubiniecki, A. S., *Large Scale Mammalian Cell Culture Technology*, Marcel Dekker, Inc., New York, 1991.
68. Volkman, L. E., *Science*, 1995, **269**, 1834.
69. Davis, A. H., *Biotechnology*, 1995, **13**, 1046.
70. Overton, L. K. and Kost, T. A., in *Baculovirus Expression Systems and Biopesticides* (eds Shuler, M. L., Wood, H. A., Granados, R. R. and Hammer, D. A.), 1995, pp. 233–242.
71. Holzman, D., *Nature*, 1995, **61**, 567.
72. Wickham, J. J. and Nemerow, G. R., *Biotechnol. Prog.*, 1993, **9**, 25–30.
73. Jobses, I., Martens, D. and Tramper, J., *Biotechnol. Bioengg.*, 1991, **37**, 484–490.
74. Tramper, J. and Vlak, J. M., *Adv. Biotechnol. Process*, 1988, **7**, 199–208..
75. Tramper, J., Smith, D., Straatman, J. and Vlak, J. M., *Bioprocess Engg.*, 1988, **3**, 37–41.
76. Cotter, T. G. and Al-Rubeai, M., *TIBTECH*, 1995, **13**, 150–155.
77. Thiem, S. M., Du, X., Quentin, M. E. and Berner, M. M., *J. Virol.*, 1996, **70**, 2221–2229.
78. Lu, A. and Carstens, E. B., *Virology*, 1991, **181**, 336–347.
79. Lu, A. and Miller, L. K., *J. Virol.*, 1996, **70**, 5123–5130.
80. Konda, A. and Maeda, S., *J. Virol.*, 1991, **65**, 3625–3632.
81. Lee, S. Y., Qu, X., Chen, W., Poloumienko, A., MacAfee, N., Morin, B., Lucarotti, C. and Krause, M., *J. Gen. Virol.*, 1997, **78**, 273–281.
82. Miller, L. K., *J. Invertebr. Pathol.*, 1995, **65**, 211–216.

ACKNOWLEDGEMENTS. I thank Dr S. P. Singh, Project Director, Project Directorate of Biological Control, Bangalore for providing necessary facilities and Dr V. V. S. Suryanarayana, Senior Scientist, IVRI, Bangalore for critically going through the manuscript.

Received 11 December 1997; revised accepted 1 April 1998