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The inhibitor of apoptosis gene (*iap-3*) of *Anticarsia gemmatalis* multicapsid nucleopolyhedrovirus (AgMNPV) encodes a functional IAP

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Summary. Programmed cell death or apoptosis is one of the defense mechanisms used by insect cells in response to baculovirus infection. Baculoviruses harbour antiapoptotic genes to prevent apoptosis and to maintain the normal course of infection. In this work, we showed that, like other baculoviruses, *Anticarsia gemmatalis* multicapsid nucleopolyhedrovirus (AgMNPV) has a functional inhibitor of apoptosis gene (*iap-3*). The *iap-3* gene was cloned, sequenced and its transcription confirmed by RT-PCR. The putative *iap-3* gene of the baculovirus AgMNPV has 864 nucleotides and codes an ORF of 287 amino acids. We have found two BIR motifs (baculoviral iap repeats) at the amino-terminal region and a carboxi-terminal RING finger motif. The IAP-3 protein of AgMNPV is closely related to IAP-3 proteins of baculoviruses and lepidopteran IAPs, with most amino acid identity (75%) with the IAP-3 protein of C*fDef*NPV (*Choristoneura fumiferana* DEF nucleopolyhedrovirus). Transcriptional analysis of the AgMNPV *iap-3* gene showed that *iap-3*-specific transcripts could be detected early and late in the infection. The *iap-3* gene of AgMNPV was shown to encode a functional IAP since insect cells transfected with increasing amounts of a plasmid containing the *iap-3* of AgMNPV showed increased resistance to apoptosis induced by a AgMNPV mutant virus.

Introduction

Baculoviruses are the most studied group of insect viruses. They first attracted attention due to their potential as biological inseticides [21]; then, as heterologous expression vectors for proteins in insect cells or larvae [15] and, more recently, as vectors for gene delivery to human cells [32]. They show two phenotypes during

infection, the occluded and budded virus (OV and BV, respectively). The first type is involved in virus dissemination through the environment, whereas the second type is related to the cell-to-cell spread of the virus inside its host [37]. The pattern of gene expression in these viruses can be temporally divided into three categories: 1. Early genes are expressed before DNA replication and are associated with the trans-activation machinery of viral genes [13]; 2. Late genes are expressed upon viral DNA replication, and 3. Very late genes are responsible for occlusion body formation [17].

Upon a viral infection, there are several types of cellular responses that may take place to prevent viral replication. One of these mechanisms is programmed cell death or apoptosis. It is a natural process that promotes homeostasis of multicellular organisms by the elimination of damaged or harmful cells and by playing an important role in tissue development and insect metamorphosis [2]. The morphological changes that occur in an apoptotic cell are well known [16]. It is a genetically controlled process in wich any mistake in its regulation can be associated to several human diseases, such as cancer, neurodegenerative disorders and autoimmune related diseases. The apoptotic cascade leading to cell destruction can be divided into a signaling and an effector phase. Cell destruction is executed by a group of cysteine-aspartate dependent proteases, called caspases [10].

Viruses can have counter-measures against apoptosis. Small genomic viruses such as RNA viruses and small DNA viruses can replicate very quickly in an apoptotic environment and large genome viruses can carry antiapoptotic genes to prevent apoptosis [33, 34]. For instance, the baculoviruses have two types of proteins that inhibit apoptosis: the P35 protein [7] and the inhibitor of apoptosis (IAP) proteins. P35 is found only in baculoviruses and it inhibits caspase activity, blocking apoptosis in its effector phase [5]. The IAP proteins were first discovered in baculoviruses [11, 4], and today, they consist of a well characterized family of genes that contain specific motifs, and being found in various animal species, including insects, nematodes and humans [9, 3]. These proteins have one to three consensus "amino-terminal" regions that have been named BIR (baculoviral iap repeat) sequence. These seem to be essential to anti-apoptotic activity [36, 20]. IAPs also have a "carboxi-terminal" zinc-binding motif named ring finger, which appears to be involved in ubiquitination of the IAP itself [39] and potentially, any caspase bound to it [39, 38, 3]. It has been shown that IAP proteins are capable of inhibiting apoptosis triggered by several stimuli, like UV radiation, chemical reagents, and the expression of *Drosophila* pro-apoptotic genes [8, 36].

The baculovirus AgMNPV has been employed to control the velvet bean carterpillar *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in Brazil for more than twenty years [21]. This baculovirus is the world's most successful viral insecticide in use, with a treated area of more than 2 million hectares of soybean crops ("F. Moscardi, personal communication"). Although highly used, little is known about the AgMNPV genes, its molecular biology and host interactions [24, 30, 27, 26, 25, 22, 23, 31]. Therefore, in this work, we have located, cloned, sequenced and analysed the transcription of the *iap-3* gene of AgMNPV during

infection. Furthermore, we have demonstrated the anti-apoptotic activity of the encoded protein.

Materials and methods

Cells and virus

A. gemmatalis (UFL-AG-286) [29] cells were grown in TC-100 medium (Gibco-BRL) supplemented with 10% fetal calf serum at 27 °C. This cell line served as host for the *in vitro* propagation of AgMNPV and the mutant vApAg [30].

Location of the iap-3 of AgMNPV

In order to amplify part of the *iap-3* gene of AgMNPV we performed a PCR reaction with 50 ng of AgMNPV DNA and the primers OpIAPF (5′-GGG TTC TAC TAC TTG GG-3′) and OpIAPR (5′-GCG CAC TTG CCG CAC GC-3′) to a final concentraction of 10 μM. They anneal at conserved BIR (nucleotides +124 and +140 relative to the *Orgyia pseudosugata* nucleopolyhedrovirus, O*p*MNPV, *iap-3* start codon) and ring finger (nucleotides +721 and +737 relative to the O*p*MNPV *iap-3* start codon) motifs, respectively (Fig. 2). The PCR program is available upon request.

The 613 bp fragment obtained from the PCR reaction was cloned into the vector pGEM-T following the manufacturer's instructions (Promega) and used to transform DH5-α *E. coli* competent cells (Life Technologies). The positive clones were sequenced (ABI-PRISM).

A non-radioactive probe was prepared using the PCR DIG Probe Synthesis Kit (Roche) using the AgMNPV DNA (50 ng) and the same primers described above. The non-radioactive probe was used to hybridize onto a nylon membrane containing DNA from a genomic AgMNPV library (*Hin*d III fragments cloned into the pGEM3Z plasmid, Promega) [19]. In brief, the selected clones containing the AgMNPV *Hin*d III library fragments were digested with *Hin*d III, separated in a 0.8% agarose gel through electrophoresis [28] and the DNA was blotted onto a nylon membrane (Hybond N+, Amersham) using the Hybaid Vacu-Aid apparatus (Hybaid). Detection was carried out using the DIG DNA labeling and detection kit (Boehringer Mannheim).

The AgMNPV fragment that hybridized with the probe (the 16.44 kb *Hin*d III-B from 15.69 to 28.06 map units, 33) was further digested with *Hin*d III and *Pst* I restriction enzymes following the manufacturer's instructions (Invitrogen), separated by electrophoresis through an 0.8% agarose gel, transferred to a nylon membrane and hybridized with the *iap-3* probe as described previously (data not shown). The fragments that hybridized with the probe (5.2 and 3.5 kb, respectively) were then cloned into the plasmid pBluescript SK+ (Stratagene) and partially sequenced (ABI-PRISM).

Sequence analysis

The sequences were analyzed using the open reading frame finder (ORF finder) and BLAST programs [1] at the NCBI home page (http://www.ncbi.nlm.nih.gov) and Prosite at the Predict Protein, Structure and Sequence analysis Pôle Bio-Informatique Lyonnais (http://pbil.univ-lyon1.fr/).

Cloning of the iap-3 of AgMNPV

We designed the primers IAPAgFSalI (5′-CGT GTC GAC ACA CAC AAT G-3′) and IAPAgRNCOI (5′-CCT CCA TGG CTG AAC G-3′) used them in a PCR reaction for the amplification of the *iap-3* gene (Fig. 2). The PCR fragment obtained from the PCR reaction

was cloned into the vector pGEM-T following the manufacturer's instructions (Promega) and used to transform DH5-α *E. coli* competent cells (Life Technologies). Positive clones (pGEMIAPAg) were then sequenced and the sequences were analyzed as described above.

Transient anti-apoptotic activity

In order to analyse the anti-apoptotic activity of the *iap-3* gene of AgMNPV, we amplified the AgMNPV *ie-1* gene promoter using 50 ng of AgMNPV DNA and the primers prIE1-459 (5′-GCT ATG CAC GCG CAA TCC G-3′) and promHind III (5′-CCC CAA GCT TGA ATT GTC GGT GAG CGT TGC GTT GCG CGT-3′) which anneal at positions −459 to −441 and −1 to −29 nucleotides relative to the start codon of the *ie-1* gene of AgMNPV (Genbank accession number AF368905), respectively. The primer promHind III also had a *Hin*d III site at the 5′ region (underlined sequence). The PCR fragment was then cloned into the vector pGEM-T easy (Promega) and sequenced. The *ie-1* promoter was removed from the recombinant plasmid pGEMprIE1 by digestion with *Eco* RI and *Hin*d III and cloned into the plasmid pBluescript SK+ (Stratagene) digested with the same restriction enzymes [28]. The resulting plasmid (pBSprIE1) was then used for the cloning of the *iap-3* gene of AgMNPV into the *Sal I* restriction site. The resulting plasmid was called pBSpIE1AgIAP3.

UFL-AG-286 cells were seeded into 6-well plates (0.5×10^6cells/well) and incubated for 24 h at 27 °C. Different amounts (2, 5 and 7 μg) of the plasmid pBSpIE1AgIAP3 were then transfected into the cells using Cellfectin (Invitrogen), following the manufacturer's instructions. Twenty-four h after transfection, the cells were infected with the mutant virus vApAg [30] with a multiplicity of infection (MOI) of 5 and incubated at 27 °C. After 1 h, the virus inoculum was removed and fresh medium added (zero h p.i.). We used UFL-AG-286 cells transfected with the plasmid pBluescript (with no insert) and infected with vApAg as a control, using the same conditions described before. Furthermore, UFL-AG-286 cells infected with vApAg and mock-infected as positive and negative controls for apoptosis, respectively. At 48 h p.i., cells were observed in an inverted light microscope (Olympus), collected, centrifuged at 1,000 × g/5 min, resuspended in PBS (pH 6.2) and incubated with trypan blue stain (0.04%). Viable cells were counted in a hemocytometer and the experiment was repeated 3 times.

For analysis of cellular DNA fragmentation, UFL-AG-286 cells that were transfected with 2 μg of the plasmid pBSpIE1AgIAP3 and infected with vApAg. At 48 h p.i., the cells were centrifuged at 1000 × g/10 min and the supernatant at 10000 × g/15 min. The same method was carried out with the cells of the negative (UFL-AG-286 cells) and positive (UFL-AG-286 cells infected with vApAg) controls. The cells and/or apoptotic bodies were treated with lysis buffer (Tris 100 mM, pH 7.5; EDTA 2.5 mM; Triton X-100 0.2%) for 1 h, at room temperature, and the total DNA was extracted with phenol/chloroform/isoamyl alcohol, precipitated with ethanol and treated with RNase (50 μg/ml). The samples were subjected to electrophoresis on 1.5% agarose gel.

Transcription analysis

A six-well plate was seeded with UFL-AG-286 cells (1×10^6/well) and incubated for 1 h at room temperature. The medium was then removed and the cells were infected with AgMNPV at a MOI of 20. After 1 h, the virus inoculum was removed and fresh medium was added (zero h p.i.). At various times p.i. (1, 3, 6, 8, 15 and 24 h p.i.), the cells were harvested and total RNA extracted using the TRIzol reagent (Invitrogen), following the manufacturer's instructions. Purified total RNA was used for the construction of cDNA using the poly-A specific primer T1 (5′-CCT GCA GGA TCC TTA GGT TTT TTT TTT TTT TT-3′) [27]. The cDNA synthesis was performed using the Mu-MLV reverse transcriptase (Invitrogen) according to the manufacturer's instructions. The PCR reaction was performed using the T2 (5′-CCT GCA

GGA TCC TTA GGT T-3′) and iapseq (5′-GTC GAG CAA CCC GC-3′) primers. The primer iapseq anneals at the positions +676 to +689 relative to the ATG codon of the *iap-3* gene (Fig. 2), and the sequence of primer T2 is identical to the first 17 nucleotides present in the primer T1 used for the cDNA reaction [27]. The fragment amplified was cloned into the pGEM-Teasy vector (Promega) and sequenced.

Results

Localization and cloning of the AgMNPV iap-3 gene

Using primers designed for specifics regions of the OpMNPV *iap*-3 gene and the baculovirus AgMNPV genomic DNA as template, we have amplified a 613 bp DNA fragment (data not shown). This fragment was cloned into the pGEM-T vector (Promega), and then sequenced (data not shown). Sequence analysis of this fragment showed a high degree of identity with insect and baculoviral inhibitor of apoptosis genes (data not shown). This PCR product was used as a probe in a Southern-blot in order to locate the *iap* gene in a genomic library of AgMNPV *Hin*d III fragments [33]. The probe hybridized with the *Hin*d III-B (from 15.69 to 28.06 m.u.), *Pst*-I J (19.66 to 23.49 m.u.) and *Pst*-I M (23.49 to 26.20 m.u.) fragments of the AgMNPV genome (Fig. 1). The two *Pst* I fragments were cloned into the pBluescript SK+ vector (Promega) and the sequence of the *iap* gene of AgMNPV was obtained from partially sequencing the two fragments (Fig. 2).

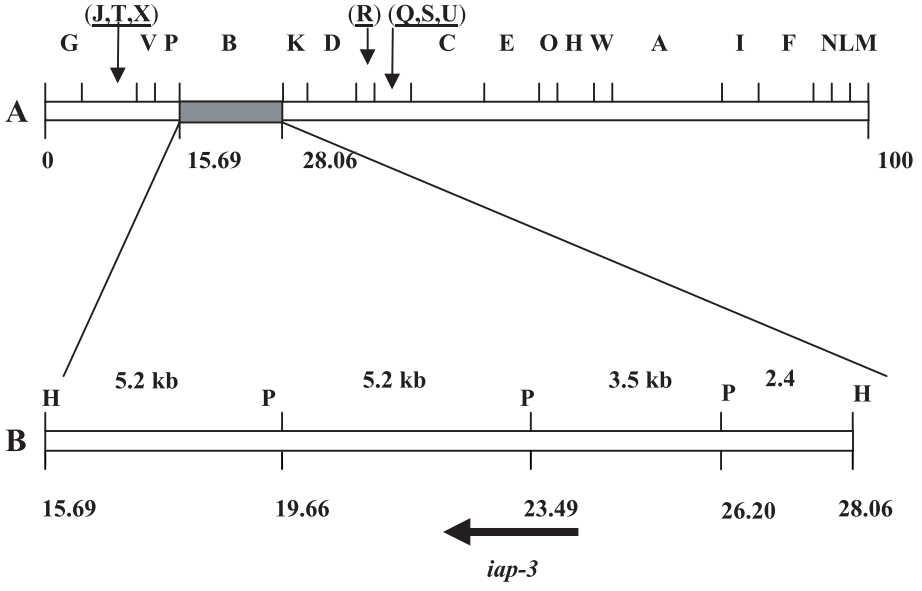

Fig. 1. A Diagram of the linearized physical map of the 133 kb A*g*MNPV genome with the restriction enzyme *Hin*d III, according to [19]. The shaded box shows the region detected by the *iap-3*-specific probe. This 16.4 Kb fragment (*Hin*d III-B), present in the pH_3B plasmid [19], was double-digested with *Hin*d III and *Pst* I and the two fragments of 3.5 and 5.2 kb were cloned into pBluescript and partially sequenced. **B** A more detailed view of the *Hin*d III-B fragment showing the position of the *Hin*d III and *Pst* I restriction sites. The arrow below diagram B shows the position and direction of the putative *iap-3* gene of A*g*MNPV. In **A** and **B** the numbers below the linear map indicate the position of the restriction sites in map units (m.u.)

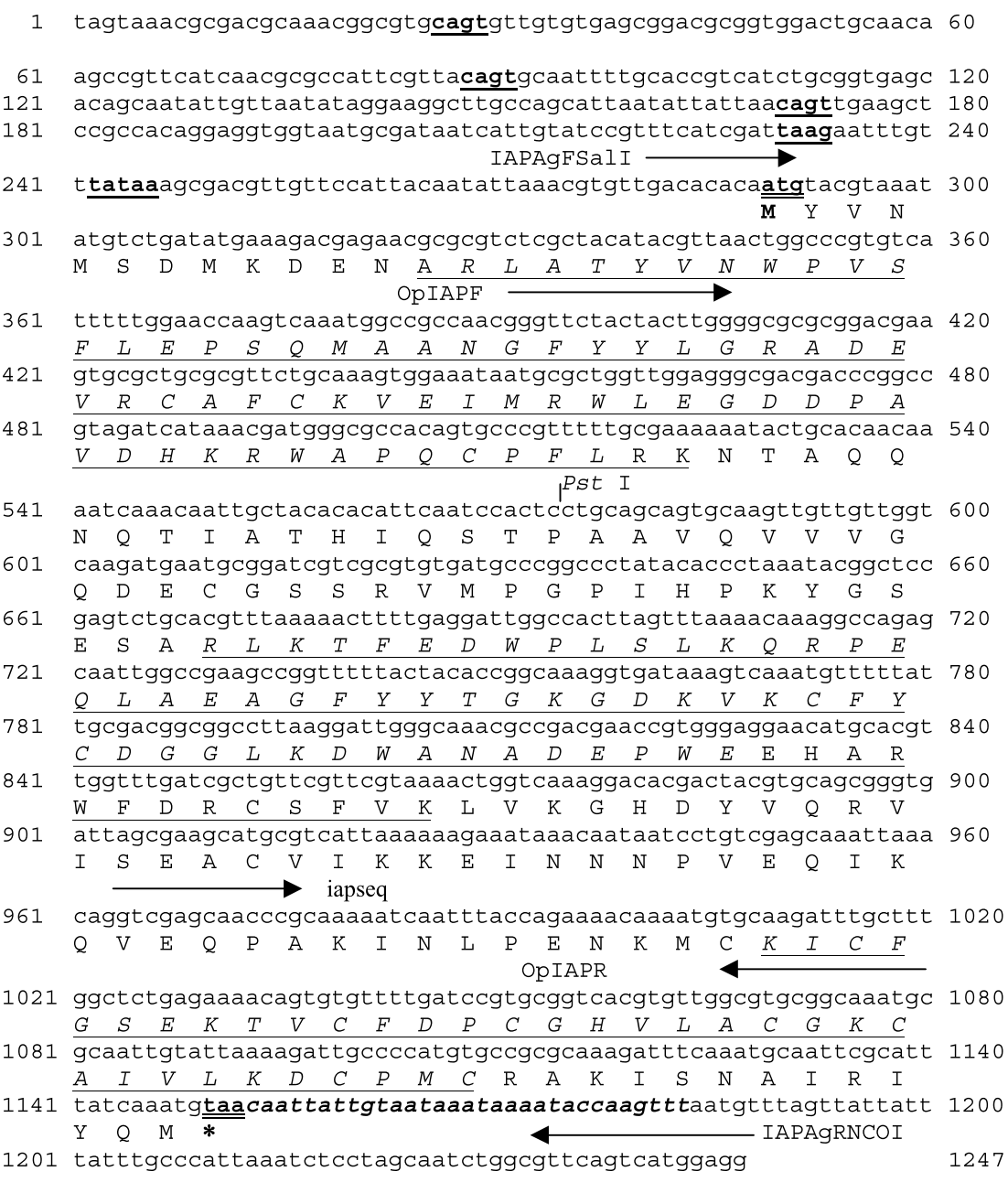

Fig. 2. Nucleotide sequence of 1247 bp containing the *iap-3* gene region from A*g*MNPV and predicted amino acid sequence of the IAP-3 protein. Putative regulatory sequences (TATA box, baculoviruses consensus early, CAGT, and late promoter, TAAG, motifs) are underlined and in bold. The start and stop codons are double underlined, the polyadenylation signal and the 3′ UTR are in bold and italics. * = stop codon. The oligonucleotides used in this work are indicated by their names and arrows above the sequence. The amino acids of the two N-terminal BIR and the C-terminal Ring finger motifs are in italics and underlined. The location of the restriction site for the enzyme *Pst* I is also shown

Sequence analysis

We have sequenced 1247 bp from the *iap-3* region of AgMNPV (around 23 m.u.). Regulatory motifs (TATA-box, CAGT and TAAG) motifs have been found upstream of *iap-3* ORF (Fig. 2). The putative *iap-3* gene of the baculovirus AgMNPV has 864 nucleotides and codes for an ORF of 287 aminoacids. We have found two BIR motifs at the amino-terminal region and a carboxi-terminal ring finger motif (Fig. 2). We also have found 2 putative N-glycosylation sites (amino acids 4 to 7, NMSD, and 85 to 88, NQTI), 1 cAMP- and cGMP-dependent protein kinase phosphorylation site (amino acid 78 to 81, RKNT), 5 Protein kinase C phosphorylation sites (110 to 112, SSR, 126 to 128, SAR, 138 to 140 SLK, 154 to 156, TGK, 246 to 248, SEK), 2 Casein kinase II phosphorylation sites (24 to 27, SFLE, 131 to 134, TFED), 3 N-myristoylation sites (104 to 109, GQDECG, 123 to 128, GSESAR, 245 to 250, GSEKTV). The IAP-3 protein of AgMNPV is more closely related to IAP-3 proteins of baculoviruses and lepidopteran IAPs, with the greatest amino acid identity to the IAP-3 protein of C*fDef*NPV (Table 1).

Table 1. Percentage identity at the amino acid level of baculovirus IAP-3 proteins and lepidopteran IAPs. The data presented were obtained using the blast program at the NCBI home page (http://www.ncbi.nlm.nih.gov/)

Virus[a] or insect[b]	IAP-3A*g*MNPV
C*fDef*NPV	75
C*f*MNPV	58
H*ycu*NPV	57
E*ppo*NPV	56
Spodoptera frugiperda	56
Bombyxmori	56
O*p*MNP*V*	55
Trichoplusia ni	55
B*usu*NPV	53
C*p*GV	52

[a]Abbreviation of baculoviruses and their GenBank accession numbers: C*f*MNPV = *Choristoneura fumiferana* nucleopolyhedrovirus: NP_848342; O*p*MNPV = *Orgyia pseudosugata* nucleopolyhedrovirus: NP_046191; B*usu*MNPV = *Bussura supressaria* nucleopolyhedrovirus: AAC34373; H*ycu*NPV = *Hyphantria cunea* nucleopolyhedrovirus: BAC55952; EppoMNPV = *Epiphyas postvittana* nucleopolyhedrovirus: NP_203195; C*fDef*NPV = *Choristoneura fumiferana DEF* nucleopolyhedrovirus: NP_932639; C*p*GV = *Cydia pomonella* Granulovirus: NP_148801

[b]GenBank accession numbers for the lepidopteran IAP-3 proteins: *Spodoptera frugiperda:* AAF35285; *Bombyx mori*: AAN46650; *Trichoplusia ni*: AAF19819

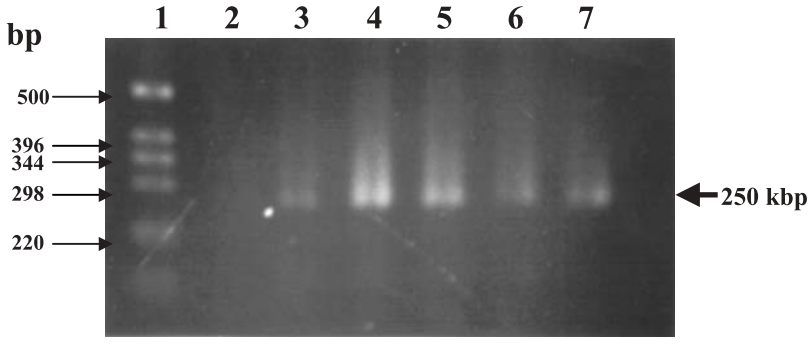

Fig. 3. Transcription analysis of the A*g*MNPV *iap-3* gene by RT-PCR. The figure shows a 0.8% agarose gel with the amplification products from RT-PCR analysis using primers T2 and iapseq as described in Materials and methods. The cDNA was amplified from total RNA preparations isolated at 1, 3, 6, 8, 15 and 24 h p.i. from A*g*MNPV-infected UFL-AG-286 cells. *1*, 1 Kb DNA ladder (Invitrogen); *2* to *7*, RT-PCR products from total RNA extracted at 1, 3, 6, 8, 15 and 24 h p.i., respectively. A fragment around 250 bp was amplified from 3 to 24 h p.i.

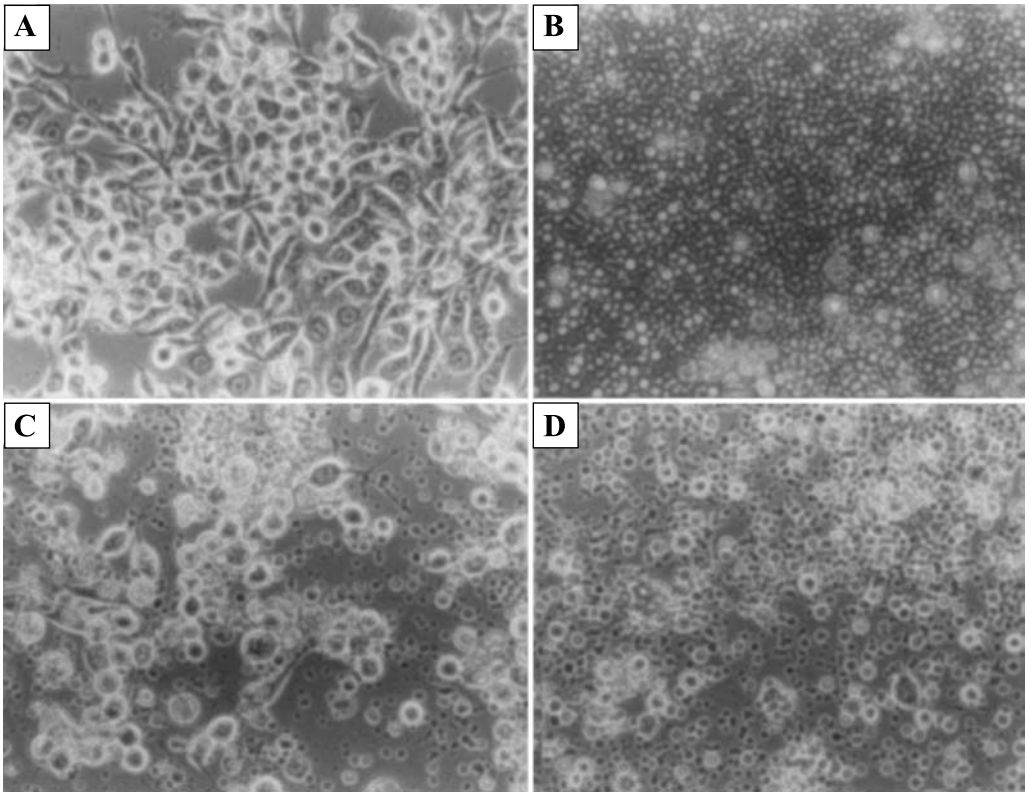

Fig. 4. Anti-apoptotic assay: **A**, UFL-AG-286 cells mock infected; **B**, UFLAG cells infected with the mutant virus vApAg (48 h p.i.); **C**, UFLAG cells transfected with the plasmid pBSpIE1AgIAP3 and infected with vApAg (48 h p.i.); **D**, UFLAG cells transfected with the pBluescript plasmid (Stratagene) and infected with vApAg (48 h p.i.)

Transcription analysis

UFL-AG-286 cells were infected with AgMNPV and at various times p.i. (1, 3, 6, 8, 15 and 24 h p.i.), RNA was extracted and used for a RT-PCR reaction for the detection of the *iap-3* transcript. We used a primer specific for the poly-A tail and an internal primer (iapseq) that anneals at the positions +676 to +689 relative to the ATG codon of the *iap-3* gene (Fig. 2). We detected the presence of a fragment of around 250 bp from 3 to 24 h p.i., showing that the *iap-3* gene is being expressed in infected insect cells from the early to the very late phase of AgMNPV expression (Fig. 3). The amplified fragment was cloned and sequenced (not shown). The sequence revealed that the end of the 3′ UTR region of the mRNA of the *iap-3* gene, before of the poly-A tail, has 31 nucleotides (Fig. 2).

Transient anti-apoptotic activity

In order to assess the anti-apoptotic activity of the *iap-3* of AgMNPV, we amplified the entire *iap-3* ORF and cloned it into a plasmid containing the AgMNPV *ie-1* promoter (not shown). This plasmid (pBSpIE1AgIAP3) was used for the anti-apoptotic assays. UFL-AG-286 cells were transfected with 2, 5 and 7 μg of the plasmid pBSpIE1AgIAP3 and at 24 h p.t., the cells were infected with the mutant virus vApAg (MOI of 5) and incubated for more 48 h at 27 °C. We detected decreasing amounts of apoptotic cells in plates transfected with increasing amounts of the plasmid (Fig. 4 and Table 2). The increase in cell

Table 2. Anti-apoptotic activity of the *iap-3* of A*g*MNPV. Percentage of viable *A. gemmatalis* cells (UFL-AG-286) transfected with increasing amounts of the plasmids pBSpIE1AgIAP3 and pBluescript (2, 5 e 7 μg) and infected (24 h p.t.) with the mutant vApAg virus (MOI of 5). The cells were analysed at 48 h p.i.

	Cell viability (mean percentage[a])	Standard deviation
UFL-AG-286 + vApAg	7.30%	±0.29
UFL-AG-286 + pBluescript (2 μg) + vApAg	6.00%	±0.03
UFL-AG-286 + pBluescript (5 μg) + vApAg	4.17%	±0.01
UFL-AG-286 + pBluescript (7 μg) + vApAg	3.30%	±0.01
UFL-AG-286 + pBSpIE1AgIAP3 (2 μg) + vApAg	12.10%	±0.81
UFL-AG-286 + pBSpIE1AgIAP3 (5 μg) + vApAg	18.90%	±0.76
UFL-AG-286 + pBSpIE1AgIAP3 (7 μg) + vApAg	26.70%	±1.05

Cell viability was measured by staining the cells with trypan blue and counting viable cells in a light microscope

[a]Mean percentage of viable cells in 3 separate experiments

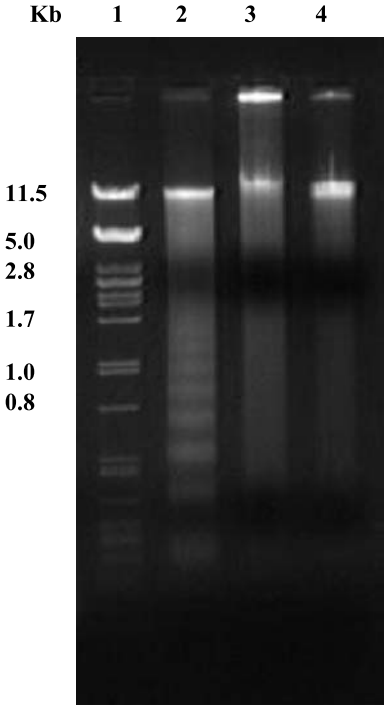

Fig. 5. DNA fragmentation analysis. Agarose gel (1.5%) eletrophoresis showing in lane *1*, DNA marker λ phage digested with *Pst* I, *2*, cells infected with vApAg 48 h p.i, positive control, *3*, cells transfected with the plasmid pBSpIE1AgIAP3 (2 μg) and infected with vApAg 48 hp.i., *4*, mock-infected UFL-AG-286 cells, negative control

viability was more than two fold (from 12.1%, with 2 μg, to 26.7%, with 7 μg). Mock-infected cells were used as a negative control with 100% cell viability and cells infected with the mutant vApAg virus, as a positive control with 7.3% of cell viability. In the plates transfected with increasing amounts of the plasmid pBluescript and infected with vApAg, we observed a decrease in the viability of the cells from 6.0% (2 μg) to 3.3% (7 μg), probably due to the cellular stress caused by the transfection with plasmid DNA. This fact shows evidence for the anti-apoptotic activity of the *iap-3* gene, since the addition of increasing amounts of pBSpIE1AgIAP3 is responsible for the increase in the viability of the cells and not simply the increasing amounts of DNA.

In order to confirm the partial inhibition of apoptosis detected, we carried out a total cellular DNA fragmentation analysis of the transfected and infected cells at 48 h p. i. The DNA from cells transfected with pBSpIE1AgIAP3 showed less DNA fragmentation when compared with the positive control (cells infected with vApAg), which presented a typical pattern of fragmentation caused by apoptosis (Fig. 5).

Discussion

Two types of apoptosis-inhibiting genes are known in the genome of baculoviruses: the *p35* and *iap* genes, and some of them have been characterized and sequenced [11, 4, 9, 12, 18, 35]. In this work, we have located, cloned, sequenced and analysed the transcription of the *iap-3* gene of AgMNPV in the course of infection.

Furthermore, we have tested the ability of the IAP-3 protein to block apoptosis induced by a mutant virus.

We have located the *iap-3* gene of AgMNPV to the *Hin*d III-B fragment, from 15.69 to 28.06 m.u. [19]. After cloning and sequencing the entire gene, we have shown that the putative AgMNPV *iap-3* gene has 864 nucleotides, which code for a protein of 287 amino acids and, more importantly, it has two well known motifs called baculoviral iap repeats (BIRs) at the N-terminal portion of the protein and a zinc finger motif called RING finger at the C-terminal which are typical of IAP proteins (Fig. 2). The AgMNPV IAP-3 protein showed more identity to the IAP-3 gene of C*fDef*NPV. All lepidopteran baculoviruses have at least one *iap* gene [18]. For instance, A*c*MNPV has three, EppoMNPV has four [18], CpGV has three, but only some of them have been shown to possess anti-apoptotic activity [18, 35]. We have located one *iap* gene in the genome of AgMNPV and it may have probably more, but this will probably come to light when the complete AgMNPV genome has its sequence resolved.

Cellular (TATA-box) and typical baculovirus early (CAGT) [13] and late (TAAG) [17] regulatory motifs were found upstream of the *iap-3* ORF (Fig. 2). We detected the transcripts of the *iap-3* gene from 3 to 24 h p.i. (Fig. 3) which is consistent with the presence of the early and late motifs in its upstream regulatory region [13, 17]. We have also shown that the *iap-3* gene of AgMNPV has an anti-apoptotic activity in transient assays (Table 2). Insect cells transfected with increasing amounts of the *iap-3* gene under the transcriptional control of the *ie-1* gene of AgMNPV were more resistant to apoptosis induced by an AgMNPV mutant. The mutant virus, known as vApAg, was isolated in our laboratory and we have shown previously that it can induce apoptosis in UFL-AG-286 cells and not in BTI-Tn5B1-4 [30, 6]. It also can, differently from the AcMNPV virus with a deletion in the *p35* gene, complete its replication in some UFL-AG-286 cells with production of occlusion bodies, despite apoptosis. Since we have looked at UFL-AG-286 cells at an early time post-infection (48 h p.i.), we did not see many polyhedra inside the cells rescued by the *iap-3* gene containing plasmid. Several baculoviruses *iap* genes are capable of blocking apoptosis induced by different stimuli, and some of them are not [8, 36].

Programmed cell death is related to the activation of caspases, a family of cysteine-aspartate dependent proteases that cleave only next to an aspartate residue and are separated in two categories: activator and effector caspases. A cascade of activation of these proteases is dependent on a pro-apoptosis signal such as a viral infection, and probably the IAP proteins interact with these proteases to block the pathway and apoptosis as well {for a recent review see [3]}. Recent studies have shown that several *iap* genes can be related to the assembly of the mitotic spindle, and citokinesis, and it is known that some virus can have *iap* genes with no known anti-apoptotic function [18, 35]. These facts contribute to the idea that the *iap* genes are not only related to apoptosis. Cellular *iaps* are larger than their viral counterparts and normally have a large spectrum of apoptosis inibition [3]. With the *iap* sequence analysis performed in our work we have shown that the AgMNPV IAP-3 protein has similar identity to most baculovirus IAP-3 proteins

and cellular *iaps*. This supports the theory according to which during the fast evolution of these viruses they managed to acquire this class of genes from the genomes of the cell they infect [14].

The reason why the mutant AgMNPV virus, known as vApAg, does not induces apoptosis in the BTI-Tn5B1-4 cells is still unclear and the type of molecular modification that this virus may have suffered has to be confirmed. We are now working to solve these questions and to elucidate some of the mechanisms of apoptosis in insect cells induced by baculoviruses.

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