Expression of the *Cydia pomonella* granulovirus *iap3* gene

D. P. Miller¹, T. Luque¹, N. E. Crook², D. Winstanley², and D. R. O’Reilly¹

¹Department of Biological Sciences, Imperial College of Science, Technology and Medicine, Imperial College Road, London, U.K.
²Horticulture Research International, Wellesbourne, Warwick, U.K.

Received August 2, 2001; accepted December 14, 2001
Published online April 26, 2002 © Springer-Verlag 2002

Summary. The IAP3 protein of *Cydia pomonella* granulovirus (CpGV) was the first identified member of the baculovirus IAP family of proteins, which have been shown to block apoptosis in diverse systems. However, little is known of the expression and subcellular localisation of CpGV IAP3 during a viral infection. This study examined IAP3 in cells infected by CpGV and in cells infected by an *Autographa californica* nucleopolyhedrovirus (AcMNPV) recombinant that carried the CpGV *iap3* gene. The levels of *iap3* specific transcripts were monitored and production of the protein was assessed using an IAP3-specific antiserum. The data showed that *iap3* is expressed during both early and late phases of infection, with a switch occurring from distal early transcription start sites to proximal late start sites. Protein levels are highest after DNA replication. IAP3 is localised exclusively in the cytoplasm. Subcellular fractionation experiments demonstrated that the protein is present in both soluble and membrane-bound cytosolic fractions. The membrane-bound fraction includes IAP3 that is associated with the mitochondria. However, the data do not support the hypothesis that release of cytochrome C from the mitochondria is involved in baculovirus-induced apoptosis.

Introduction

Insect baculoviruses encode two classes of protein that can block apoptosis, the p35 and inhibitor of apoptosis (IAP) proteins [24]. Of these the p35 proteins are best understood. The *Autographa californica* nucleopolyhedrovirus (AcMNPV) p35 gene is expressed predominantly as an early gene and transcripts are detectable from as early as 1 h p.i. The transcription of p35 peaks at around 6 h p.i. and declines thereafter, but low levels can still be detected at late times after infection [25]. The protein blocks apoptosis by inhibiting the activity of
caspases, a family of cysteine proteases thought to be central components of the apoptotic process [3, 5, 35, 37]. p35 is a caspase substrate but forms a stable complex with the enzyme, thereby inhibiting its activity in a stoichiometric fashion. Anti-apoptotic baculovirus IAP proteins act upstream of p35. They can prevent the activation of caspases, but unlike p35, cannot block apoptosis once the target caspase has been activated [28]. A series of proteolytic cleavage events are involved in caspase activation to give rise to a heterotetrameric enzyme comprising two large and two small subunits. *Orgyia pseudotsugata* nucleopolyhedrovirus (OpMNPV) IAP3 has recently been shown to inhibit activation of *Spodoptera frugiperda* caspase-1 by blocking the first activation cleavage between the large and small subunits [17]. Baculovirus IAPs interact with the *Drosophila* pro-apoptotic proteins Doom, hid, grim and reaper [11, 32, 33]. However, baculoviruses do not infect *Drosophila* and the relevance of these interactions to infection of permissive host cells is unclear. The situation is further complicated by the fact that there are several classes of baculovirus IAP proteins, and individual baculoviruses often express multiple IAPs. Many of these IAPs do not appear to block apoptosis. This was first recognized when the complete sequence of OpMNPV was determined. As noted, OpMNPV was previously known to express an active IAP [4]. The sequence analysis demonstrated that OpMNPV actually encodes four IAPs, with the known active IAP now designated OpIAP3 [1]. Baculovirus IAPs are typically named according to which OpMNPV IAP they are most homologous to. AcMNPV is known to encode homologues of OpIAP1 and 2 (designated AcIAP1 and 2 respectively) in addition to p35 [2]. It has not been possible to demonstrate a function for either of these [9]. Like OpMNPV, *Epiphyas postvittana* nucleopolyhedrovirus (EppoMNPV) has been shown to encode four IAPs [22]. However, in contrast to OpMNPV, EppoIAP2 blocks apoptosis, whereas EppoIAP1 delays it, and EppoIAP3 and 4 have no detectable anti-apoptotic activity.

We have been studying a *Cydia pomonella* granulovirus (CpGV) IAP that is known to have anti-apoptotic activity [7]. This is a member of the IAP3 family [21], and we refer to it as CpIAP3. This was the first IAP protein to be identified [7]. Much of the work to date exploring baculovirus IAP function has used transient expression systems, and relatively little is known of these proteins in the context of the normal infection process. Here, we have explored the expression and subcellular localisation of CpIAP3 in two infection systems, CpGV infection of *C. pomonella* cells, and vASB6-1 infection of *Spodoptera frugiperda* SF21 cells. Although CpGV replicates in *C. pomonella* cells in culture, it is difficult to obtain the high titre stocks of the virus that would be required for the expression analysis work described here. vASB6-1 represents an alternative system that can be used to generate these data. The virus is a p35-deletion mutant of AcMNPV that expresses Cpiap3 [7]. Infection of SF21 cells by p35-deletion mutants of AcMNPV results in extensive apoptosis. This is blocked by CpIAP3. Thus, vASB6-1-infection of SF21 cells represents a convenient model system for the analysis of CpIAP3 where it is certain the protein is actively blocking apoptosis. Our data show that CpIAP3 is expressed both early and late in infection from distinct start sites.