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Review

Reaching the melting point: Degradative enzymes and protease inhibitors involved in baculovirus infection and dissemination

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ABSTRACT

Baculovirus infection of a host insect involves several steps, beginning with initiation of virus infection in the midgut, followed by dissemination of infection from the midgut to other tissues in the insect, and finally culminating in "melting" or liquefaction of the host, which allows for horizontal spread of infection to other insects. While all of the viral gene products are involved in ultimately reaching this dramatic infection endpoint, this review focuses on two particular types of baculovirus-encoded proteins: degradative enzymes and protease inhibitors. Neither of these types of proteins is commonly found in other virus families, but they both play important roles in baculovirus infection. The types of degradative enzymes and protease inhibitors encoded by baculoviruses are discussed, as are the roles of these proteins in the infection process.

Contents

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Introduction

Members of the virus family *Baculoviridae* contain large double-stranded DNA genomes (80–180 kbp) and infect invertebrates in the class Insecta. Like other DNA viruses, baculovirus genomes contain many genes that have easily recognizable homology with host genes, indicating gene transfer from the genomes of their hosts. The function of these host-derived genes benefits baculovirus infectivity, virulence, and their ability to disperse to other susceptible hosts. It has been hypothesized that at least for some genes, once the insect-derived gene is part of the virus genome, its function evolves to optimize virus infection or expand the virus host range (Lung and Blissard, 2005).

There are many unusual and interesting features associated with baculoviruses (Clem and Passarelli, 2013). One of these is the existence of several types of baculovirus genes that encode degradative enzymes, which is uncommon amongst viruses. These enzymes facilitate baculovirus infection of insects through several processes, including penetration of the peritrophic matrix, a layer that protects epithelial cells in the insect gut, to establish primary infection; melanization, or darkening of tissue; and liquefaction of the infected cadaver at late stages of baculovirus infection. In addition, baculoviruses are one of only two virus families, the other being the poxviruses, which commonly encode protease inhibitors. We discuss the functions of two types of baculovirus-encoded protease inhibitors in curtailing innate defense mechanisms. Each of the more than seventy baculovirus genomes sequenced to date contains various combinations of these degradative enzyme and protease inhibitor genes. We review the functions of these proteins in virus infection and how viral pathogenesis may differ in viruses carrying different sets of degradative enzymes or protease inhibitors. Finally, we discuss the interplay amongst these gene products and how they ultimately cooperate to allow efficient virus replication and spread. The review does not cover viral-encoded nucleases.

Baculovirus replication and pathogenesis

Only about two-thirds of the approximately 150 genes encoded by the prototype baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) have been characterized enough to know their specific function (Rohrmann, 2013). Approximately 15% encode proteins dedicated to gene transcription and nucleic acid replication and processing. Around 33% of the virally-encoded proteins have been identified as virion-associated proteins using proteomic methods (Hou et al., 2013). When 141 open reading frames (ORFs) were systematically deleted in Bombyx mori NPV (BmNPV), 86 of the mutant viruses were still able to replicate normally in cell culture (Ono et al., 2012), indicating that over half of baculovirus genes encode proteins that are either necessary for in vivo infection or serve auxiliary functions such as enhancing virulence or virus transfer between hosts. These numbers provide a glimpse into the diversity of functions and potential strategies that allow these viruses to outmaneuver their hosts.

The family *Baculoviridae* contains four recognized genera, *Alphabaculovirus*, nucleopolyhedroviruses (NPVs) that replicate in lepidopteran hosts; *Betabaculovirus*, granuloviruses (GVs), which also have lepidopteran hosts; *Gammabaculovirus*, NPVs that replicate in hymenopteran hosts, and *Deltabaculovirus*, which contains a single member that replicates in a dipteran host (Jehle et al., 2006). The host range of each baculovirus can vary from one to several dozen insect species (Harrison and Hoover, 2012), and gene acquisition has undoubtedly been instrumental in allowing infections in new hosts.

Baculovirus infection commences when an insect consumes vegetation contaminated with viral occlusion bodies (OBs), which consist of occlusion-derived virions (ODV) encased in an environmentally stable matrix. The proteinaceous component of the OB is mainly composed of a single protein, polyhedrin (called granulin in betabaculoviruses), that is abundantly synthesized at very late stages of virus replication. Depending on the virus, an OB contains between one and around 100 enveloped ODV. Once the OB reaches the midgut, the protective matrix is dissolved in the alkaline pH of the midgut lumen, releasing the ODV. To access host cells, the ODV must cross a barrier lining the midgut called the peritrophic matrix, a layer consisting of chitin and proteins that separates the midgut epithelium from the gut lumen. As discussed below, some viruses produce enhancins, metalloproteases that degrade the peritrophic matrix. ODV enter midgut epithelial cells by membrane fusion and this event requires a number of structural factors called per os infectivity factors (PIFs) to mediate successful virion attachment and entry. ODV replicate in the nucleus of midgut cells, and in most baculoviruses, a second type of enveloped virion is produced called budded virus (BV). The name BV reflects the fact that these virions acquire their envelopes and associated glycoproteins as they bud from the cellular plasma membrane. Infection can be restricted to midgut cells (in the case of deltabaculoviruses, gammabaculoviruses and a betabaculovirus), but for most baculoviruses, BV escape the midgut and extensively infect other tissues in the insect, where both BV and ODV are produced. Thus, the two virion forms have distinct functions during infection: BV spread infection between cells within an infected insect, while ODV are formed in the nucleus of infected cells, embedded in OBs, and released into the environment upon death of the host, spreading infection horizontally between insect hosts.

In the case of most baculoviruses, at the end of the replication cycle the insect cuticle becomes melanized (darkened) and the insect cadaver liquefies (Slack et al., 1995; Hawtin et al., 1997; Kang et al., 1998; Wang et al., 2005). The complete dissolution of the infected insect allows the stable OBs to be efficiently released so they can be ingested by other susceptible feeding insects, initiating another infection cycle. Insect melanization and liquefaction requires the viral degradative enzymes chitinase and cathepsin, which work together in liberating OBs from the dead larvae. In viruses that do not establish a systemic infection, the mechanism for OB release is not clear but, as addressed below, the presence of other degradative enzymes may be involved in alternative mechanisms of release.

Baculovirus-encoded degradative enzymes

Chitinase

Chitinases (EC 3.2.1.14) degrade chitin, a β-1,4-linked N-acetylglucosamine (GlcNAc) homopolymer, which constitutes an integral component of the exoskeleton, peritrophic matrix, certain organs (salivary glands and trachea), muscle joints and eggshells of arthropods. Different types of chitin may form either hard or soft barriers, and these are used to isolate organs or the organism from invading pathogens or a harsh environment (Muthukrishnan et al., 2012). Organisms produce specialized chitinases, each with different substrate preferences (Koga et al., 1999), and some that are enzymatically inactive but which bind chitin, perhaps to assist binding and catalysis of insoluble chitin fibers by active chitinases (Jollès and Muzzarelli, 1999; Koga et al., 1999; Vaaje-Kolstad et al., 2005). Insects utilize chitinases for growth and remodeling of exoskeleton and peritrophic matrix, which are essential structural and physiological invertebrate layers, so their activity must be stringently regulated (Kramer et al., 1985). In addition, some organisms that lack chitin produce chitinases as a defense against feeding and parasitism by insects and chitinous pathogens (Jollès and Muzzarelli, 1999).

Viral chitinases are family 18 glycohydrolases that belong to a family of multi-modular proteins found in diverse organisms including mammals, bacteria, plants, and fungi (Jollès and Muzzarelli, 1999).