INTRODUCTION

Pathogens and viruses, in particular, play an important role in the population dynamics and maintenance of genetic polymorphism of their hosts (Hamilton et al., 1990; Antonovics and Thrall, 1994). Consequently, latency of viruses including human viruses is a widespread and actively studied phenomenon (Alberts et al., 1994; Bakhov et al., 2003; Boots et al., 2003). After exposure to stress, latent viral infection can enter the acute phase, which can cause a disease or death of virus carriers.

Insects are the most abundant group among all known species, and baculoviruses are widespread in insects experiencing population explosions (Gulli and Rybina, 1988; Il’inykh et al., 1995; Kukan, 1999; Milks and Myers, 2001). Hence, baculoviruses and their hosts can be a convenient model to study the pathogen–host interactions. However, latent viruses are common in insect populations in addition to exogenous viruses (Eastwell et al., 1999; Burden et al., 2003; Cooper et al., 2003; Il’inykh et al., 2004). Hence, identification of the effect of latent and exogenous viruses during acute infection and after insect death is problematic without reliable techniques for the detection of latent infection. Differentiation of these effects on the infection progress is of fundamental importance since insect response to latent viral infection is largely determined by the physiological status of the virus and by stress factors. Consequently, insect response to exogenous infection is, along with the physiological status of the virus, determined by its biological activity and multiplicity of infection.

In this context, latency of baculoviruses remained enigmatic in many respects for a long period of time, although spontaneous polyhedral disease was first described in insects over a hundred years ago (Podgwaite and Mazzone, 1986). The progress in techniques of molecular biology and viral culturing in vitro and in vivo, ruling out exogenous viral infection of cell lines and insect cultures, in the last two decades made possible a number of fundamental achievements in latent virus detection and understanding the mechanism of latent infection. The obtained data largely extend our knowledge about latency in the system of interactions between baculoviruses and their insect hosts at different ecological levels.

Abstract—Due to their widespread natural occurrence, baculoviruses and their insect hosts are a convenient model to study the pathogen–host interactions. However, the absence of reliable techniques for the detection of latent viral infection, which is common in insect populations, is among the constraints of such studies. The recent progress in molecular biology techniques made it possible to obtain the fundamental data on the detection of latent viruses in different insect species as well as on the mechanism of latent infection induction, which are reviewed below. The obtained data in many respects expand our knowledge about the role of latency in the system of interactions between baculoviruses and their insect hosts at different ecological levels.

OVERCOMING VIRAL INFECTION

Viral infection of larvae does not always cause insect death; some of them complete their development and leave offspring. Stairs (1965) proposed that metamorphosis can overcome the disease development considering that histolysis of infection-sensitive cells can take place during this process. Another way to overcome the infection is mediated by an age-related increase in larval resistance to baculovirus infection (Evans, 1983; Elam et al., 1990; Engelhard and Volkman, 1995). Such increased resistance to virus can be due to the developmental increase in larval weight (Evans, 1983). Alternatively, it can result from the change in midgut pH and the corresponding changes in the activity of digestive proteases (Elam et al., 1990; Milks and Myers, 2001). The age-related larval resistance to virus can be partially due to the capacity of the midgut epithelium to cast off infected cells to the midgut lumen (Engelhard and Volkman, 1995).

There is evidence of hormonal control of viral replication. In particular, viral infection in the larvae of silkworm Bombyx mori was inhibited by β-ecdysone (Hou
and Yang, 1990; cited from Hoover et al., 2002). In addition, juvenile hormone and ecdysone, which are the key factors of growth, molting, and metamorphosis in insects, can modulate the immune response. For instance, juvenile hormone III and 20-hydroxyecdysone induced immune response in silkworm, which was manifested as active development of oenocytoids and granulocytes (Han et al., 1995; cited from Hoover et al., 2002).

Consequently, viral infection can affect the hormonal balance of infected individuals. Ecdysteroid UDP-glucosyltransferase (egt) gene, whose product inactivates insect ecdysone, was found in some baculoviruses (O’Reilly and Miller, 1989; Shikata et al., 1998; Slavicek et al., 1999). For instance, infection of caterpillars of silkworm (Shikata et al., 1998) and gypsy moth Lymantria dispar (Slavicek et al., 1999) with viruses carrying the egt gene affected ecdysis and induced abnormal growth of insects manifested as a stepping over an instar or an elongated instar. Inoculation of insects with viruses carrying an egt gene with deletions induced no abnormal growth. At the same time, experiments on the infection of V instar gypsy moth with nucleopolyhedrovirus (NPV) carrying an egt gene demonstrated no significant effect of this gene on insect development (Myers et al., 2000).

Insect resistance can also be related to active immune response to viral infection. Larval cells of cabbage looper Trichoplusia ni infected by the virus were encapsulated by hemocytes and destroyed (Washburn et al., 1996).

Possible ways to overcome viral infection include apoptosis of insect cells. Recently Zhang et al. (2002) presented direct evidence for in vivo apoptosis induction in Spodoptera littura larvae infected with Autographa californica NPV. Consequently, certain baculoviruses and their hosts were shown to block apoptotic pathways involving the genes of inhibitor of apoptosis (iap) and caspase inhibitor p35 (Bump et al., 1995; Bertin et al., 1996; Manji et al., 1997).

Latency of baculoviruses can also be considered as a case of overcome acute viral infection or a compromise between the apoptotic response of the infected insect and the capacity of baculoviruses to block apoptosis. For instance, infection of Spodoptera frugiperda cells with Autographa californica NPV demonstrated that a deletion or mutation of the apoptotic suppressor gene p35 can result in a latent infection (Lee et al., 1998).

LATENT VIRUS DETECTION

By analogy with latency of temperate bacteriophages, Gershenzon (1961) proposed viral genome integration into the genetic apparatus of the host cell during latent infection. In addition, the presence of an inhibitor capable to trigger latent state of virions (Himeno et al., 1973) as well as a cell barrier blocking viral distribution and further reproduction (Tinsley, 1979) were assumed.

The integration of baculoviral and cellular genomes was first demonstrated by Kok et al. (1983) for latent infection in silkworm. They determined viral DNA quantities in cellular DNA samples from superficially intact insects by the acceleration of reassociation of radioactive iodine- or phosphorus-labeled viral DNA and unlabeled cellular DNA. According to their data, a haploid genome of silkworm pupae contained 2–3 copies of viral DNA, which did not exclude the presence of the viral genome integrated into the DNA of each cell of the insect (Kok et al., 1983).

The method of reassociation kinetics of viral DNA was also used to quantify viral nucleotide sequences in greater wax moth Galleria mellonella, Prague strain, cultivated under laboratory conditions for long period of time (Miryuta et al., 1985). The proportion of viral DNA equaled 2.0 ± 0.6% of the cellular genome (Miryuta et al., 1985). Dot hybridization was used to detect latent viral infection in gypsy moth (Il’inykh et al., 1995). Hatched caterpillars from eggs collected in their outbreaks were cultured on an artificial medium under laboratory conditions excluding exogenous viral infection. The intensity of viral DNA–gypsy moth DNA hybridization was always higher than the intensity of nonspecific hybridization of viral DNA to salmon sperm DNA. This indicated the presence of sequences similar to viral DNA in the cellular genome (Il’inykh et al., 1995). Latent viral infection was described in a cell culture of corn earworm moth Heliothis zea (Lin et al., 1999). Productive viral infection was observed for over 5 days after spontaneous viral reactivation in cell lines with less than 0.2% of infected cells. Persistently infected cells could contain up to 16% viral DNA. DNA hybridization demonstrated that viral DNA was integrated into the chromosomes of host cells (Lin et al., 1999).

Latent viral infection transmitted from one insect generation to another without symptoms and detected at all stages of the life-cycle was described in cabbage moth Mamestra brassicae (Hughes et al., 1993). While the virus remained undetectable in a laboratory culture of M. brassicae by the standard extraction techniques, the control virus-free insects died from NPV after feeding with the fat body of virus-carrying insects. Later Hughes et al., (1997) applied polymerase chain reaction (PCR) to demonstrate the presence of polyhedrin mRNA and transcription factors activating baculovirus late promoters. In addition, viral infection in caterpillars was limited to the fat body (Hughes et al., 1997). These data indirectly confirmed the persistence of a latent virus in the cabbage moth body and a low-level expression of the genes. Nevertheless, both studies on the detection of latent viral infection in insects remained the source of the virus unclear. Later experiments simulated latent viral infection in vivo and in vitro.