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Comprehensive analysis of host gene expression in *Autographa californica* nucleopolyhedrovirus-infected *Spodoptera frugiperda* cells

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ABSTRACT

Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) is the best-studied baculovirus and most commonly used virus vector for baculovirus expression vector systems. The effect of AcMNPV infection on host cells is incompletely understood. A microarray based on *Spodoptera frugiperda* ESTs was used to investigate the impact of AcMNPV on host gene expression in cultured *S. frugiperda*, Sf21 cells. Most host genes were down-regulated over the time course of infection, although a small number were up-regulated. The most highly up-regulated genes encoded heat shock protein 70s and several poorly characterized proteins. Regulated genes with the highest score identified by functional annotation clustering included primarily products required for protein expression and trafficking in the ER and golgi. All were significantly down-regulated by approximately 12 h post-infection. Microarray data were validated by qRT-PCR. This study provides the first comprehensive host transcriptome overview of Sf21 cells during AcMNPV infection.

Introduction

Baculoviruses infect insects and have enveloped rod-shaped nucleocapsids and double-stranded circular genomes that range from 80 to 180 kb in size. Their replication cycle involves a regulated cascade of gene expression in which early and delayed early virus genes are transcribed by host RNA polymerase II (RNA pol II), whereas late and very late genes are transcribed by a virus encoded RNA polymerase. Baculoviruses that infect Lepidoptera produce two morphological forms budded virus (BV), which spreads the virus from cell to cell in infected insects, and occlusion derived virus (ODV), which spreads the virus between insect hosts (reviewed in (Rohrmann, 2008)).

Baculoviruses have long been used as microbial insecticides to control insect pests in agriculture and forestry. In the 1980s, they were developed as a powerful eukaryotic protein expression vector (Summers, 2006). The best-studied baculovirus, *Autographa californica* multicapsid Nucleopolyhedrovirus (AcMNPV) is the type species of the genus alphabaculovirus in the family Baculoviridae (International Committee on Taxonomy of Viruses, 2008; Jehle et al., 2006). It is the prototype and most commonly used virus vector for the baculovirus expression vector system (BEVS). Proteins expressed optimally by BEVS can achieve levels of up to 50% or more of the total cellular protein because of the strong polyhedrin gene (*polh*) promoter, which is turned on very late in the viral infection cycle (Summers and Smith, 1987). The *polh* gene encodes for the matrix protein that embeds the ODV in large (1–5 μM) protein occlusion bodies, called polyhedra. The *polh* gene is dispensable for baculovirus infection of cultured insect cells and has been deleted from most baculovirus expression vectors (O'Reilly et al., 1992; Smith et al., 1983). The temporal regulation of the *polh* promoter has advantages for synthesizing proteins that may be cytotoxic because it acts in a similar manner to a conditional promoter, switching on very late in the infection cycle, after the virus has already replicated, assembled, and released progeny virions in the form of BV.

Since its inception, BEVS has become one of the leading platforms for eukaryotic protein expression in both academic and industrial settings. Proteins expressed in BEVS are glycosylated, phosphorylated, and acetylated at appropriate sites and in most cases are fully functional (Jarvis, 1997). BEVS has many advantages over other expression systems, such as the ability to synthesize multiple proteins for protein complexes and the production of virus-like-particles

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(VLP). A number of animal vaccines made using BEVS technology are licensed for use around the world (reviewed in (Hu et al., 2008; van Oers, 2006)). BEVS is also a robust platform for the display of expressed eukaryotic protein libraries for screening of ligands and drugs (Makela and Oker-Blom, 2006). BEVS is emerging as one of the best platforms for expressing eukaryotic integral membrane proteins for high-resolution structural studies, which is currently a major challenge in biomedical research.

However, AcMNPV infection impacts host cells in many ways that may influence the expression of heterologous proteins, particularly secretory and integral membrane proteins, which can be more difficult to express than cytoplasmic proteins using BEVS (Higgins et al., 2003; Jarvis and Summers, 1989; Tate et al., 2003; van Oers et al., 2001a). During infection, the nucleus swells, the cell rounds up, and cytoskeletal architecture is modified (Charlton and Volkman, 1991; Roncarati and Knebel-Morsdorf, 1997; Volkman and Zaal, 1990). The cell cycle arrests at G2/M in AcMNPV-infected Sf9 cells (Braunagel et al., 1998; Ikeda and Kobayashi, 1999). Host protein synthesis in AcMNPV-infected insect cells is shut down over the course of infection, beginning at approximately 10–12 hpi as the virus takes control of the cell to produce new virions (Carstens et al., 1979; Maruniak and Summers, 1981). By 24 hpi, the majority of newly synthesized proteins are either virus encoded or virus induced. The mechanism responsible for the shut off of host protein synthesis is not fully understood but appears to correlate with a reduction in host gene transcripts (Ooi and Miller, 1988; van Oers et al., 2001b, 2003). A differential display approach was used to identify potentially up-regulated host genes (Nobiron et al., 2003). In this study, among over 3000 cDNA fragments, most were down-regulated between 12 and 18 hpi. Only one host gene, heat shock protein 70 cognate (hsc70) mRNA was confirmed to be up-regulated.

To better understand the effects of AcMNPV on host cell gene expression as it relates to BEVS, we performed a more comprehensive study on the effects of AcMNPV infection on host gene expression in Sf21 cells, a widely used host cell line derived from *Spodoptera frugiperda* pupal ovaries (Vaughn et al., 1977). We used microarrays designed from an extensive *S. frugiperda* EST database, together with quantitative real time-polymerase chain reaction (qRT-PCR), to investigate the effects of AcMNPV infection on host gene transcription over time up until 48 hpi. Our results showed that transcripts for the majority of host genes declined substantially by 12 hpi. Furthermore, using Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis et al., 2003; Huang et al., 2009) to cluster the host genes we identified cellular processes and pathways that were affected by AcMNPV late in infection. The highest scoring cluster was enriched in genes involved in protein trafficking and processing in the ER and Golgi. We expect these data to be useful for optimizing BEVS for expressing foreign proteins, particularly secretory and integral membrane proteins. We also identified several up-regulated genes, in addition to hsp70.

Results

Microarray data analysis of Sf21 host genes during AcMNPV infection

To better understand the effects of AcMNPV infection on host gene expression we compared the transcription profiles of *S. frugiperda* genes over time in AcMNPV-infected Sf21 cells using microarrays. The microarrays comprised oligonucleotide (60-mer) features and were designed using available *S. frugiperda* EST data (SPODOBASE, http://bioweb.ensam.inra.fr/spodobase/ (Negre et al., 2006)). The experiment was done in quadruplicates and we employed spike-in mRNAs as internal controls. The expression levels of host genes of Sf21 cells infected with AcMNPV at 6, 12, and 24 hpi were compared to the expression levels of the same genes from the mock infection sample. The comparison was performed with the GeneSpring GX 11 software (Agilent) using one-way ANOVA method; only genes that showed significant differences in expression levels ($p<0.05$) were selected. The data showed that the numbers of host genes significantly up-regulated more than 1.2 fold decreased as the infection progressed (Fig. 1A). Although a 1.2 fold change is a low cutoff for microarrays, it was selected in order to identify changes in host gene transcript levels at 6 hpi, which were subtle compared to 12 and 24 hpi. Among host genes whose expression was up-regulated during the course of infection, approximately 66% were up-regulated at 6 hpi, whereas 18%, and 16% were found to be up-regulated at 12 and 24 hpi, respectively (Fig. 1A). On the contrary, the number of host genes down-regulated more than 1.2 fold increased as the infection progressed. Approximately 19%, 40%, and 41% were down-regulated at 6, 12, and 24 hpi, respectively (Fig. 1B). Thus the trend in up- and down-regulated genes over the time course of infection showed an inverse relationship (Fig. 1C). Because the majority of the genes were represented on the microarray by three different probes, the number of genes represented in Fig. 1 was approximated by dividing the number of probes by 3. Although most of the redundant ESTs (multiple ESTs representing the same gene) present in SPODOBASE were removed before designing the probes to print on the microarray

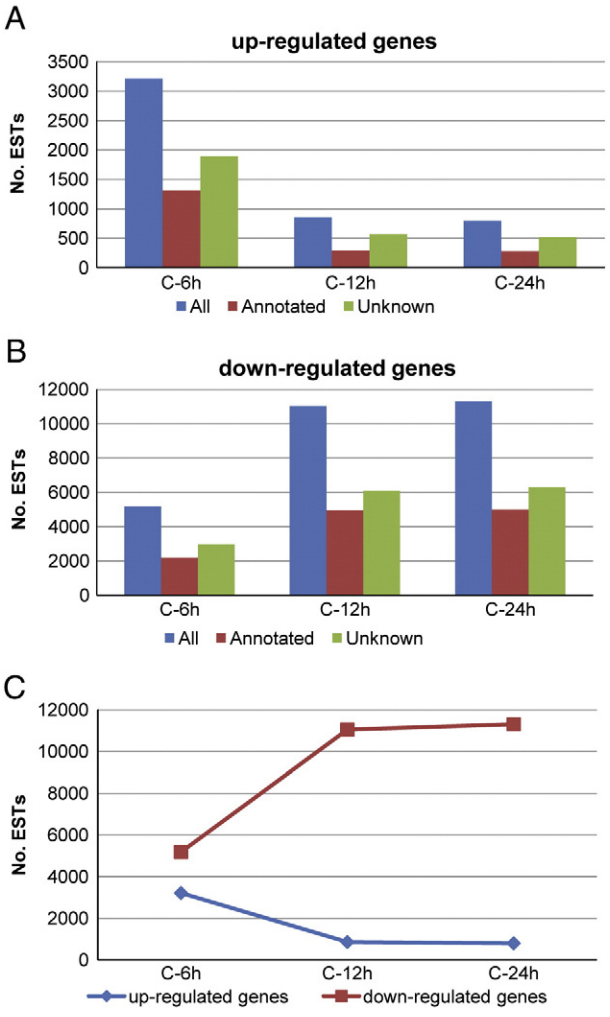

Fig. 1. Patterns of host gene expression in AcMNPV-infected Sf21 cells determined by microarray analysis. Probes for host genes designed based on ESTs sequence data from SPODOBASE, http://bioweb.ensam.inra.fr/spodobase/). The approximate number of genes that were A) up-regulated or B) down-regulated during the time course of infection (6, 12, and 24 hpi) compared with the mock-infected controls; C) Chart comparing the expression trends exhibited by up- and down-regulated genes during the time course of infection. Only genes with their level of expression equal to or more than 1.2 fold change and showed significant difference ($p<0.05$, one-way ANOVA) were selected.