increased attention and vigilence, as well as underlining the range of parasites expected in many systems.

Contributed paper. Thursday, 15:15. **241**

Bacillus thuringiensis toxins vs baculovirus: differential induction of immune system related genes in _Spodoptera exigua_

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Spodoptera exigua Hübner is a polyphagous pest native from Asia that has been spread worldwide. It is a major threat not only for field or flower crops, but also for greenhouse vegetable cultivations. To reduce losses due to *S. exigua* damage, growers often opt for biological control, such as using insecticidal products based on *Bacillus thuringiensis* Berliner (Bt) or baculovirus. Both pathogens act by ingestion and lead to insect death within few days. To counteract the infection, *S. exigua* relies on its immune system response, and production of antimicrobial peptides (AMPs) and proteins is an important part of the innate immune defense cascade triggered by pathogens. In this study, *S. exigua* transcriptome was mined for the presence of unigenes encoding for AMPs and lysozymes, resulting in the identification of a wide and diverse spectrum of these types of defense molecules. Then we compared their transcript abundance in larval midguts after ingestion of different Bt toxins (such as Cry1C and Vip3Aa) or *S. exigua nucleopolyhedrovirus* (SeMNPV) occlusion bodies. Results showed that both Bt proteins triggered a similar pattern of response, which included the specific overexpression of around 80% transcripts tested. In contrast, after SeMNPV ingestion, expression of AMPs decreased or did not change. The possible meaning of *S. exigua* physiological response to different pathogens employed in biological control is discussed.

CONTRIBUTED PAPERS Thursday, 14:00-16:00

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Contributed paper. Thursday, 14:00. **242**

Lysine residues in N-terminal tail of a viral histone H4 are crucial in controlling host gene expression

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An endoparasitoid wasp, *Cotesia plutellae*, parasitizes young larvae of the diamondback moth, *Plutella xylostella*. Parasitized larvae undergo significant immunosuppression and developmental alteration. Various parasitic factors have been identified from a polydnavirus, *C. plutellae* bracovirus (CpBV), and teratocytes. A viral histone H4 is identified from CpBV episomal genome. It encodes 141 amino acid residues and shares high sequence homology (82.5%) with host histone H4. Its extended N-terminal region (38 residues) contains 9 Lys residues. Pull-down assay showed that CpBV-H4 interacted chromatin remodeling apparatus, such as SWI/SNF complex. Subtractive suppressive hybridization showed that its expression in nonparasitized host alters the expression of various target genes classified various categories. Indeed, the viral H4 can join to a nucleosome in *in vitro* reconstruction assay. A chromatin immunoprecipitation (ChIP) assay indicates that the viral histone H4s are located at AT-rich regions near to the inducible genes, such as immune, detoxification, and metabolism. The truncated viral histone H4 loses almost inhibitory activity on host immunity. A series of truncated mutants or point mutations at Lys indicate that a specific Lys at 6^{th} from N terminal is crucial to exhibit its epigenetic control of host immunity.

Contributed paper. Thursday, 14:15. **243**

Heat-shock protein 90 is a broadly active regulator for baculovirus infection

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Cellular chaperon Hsp90 plays important roles in diverse biological processes, including signal transduction, protein folding and trafficking, etc. Many viruses, including HCV, HSV and Influenza virus, are dependent on host Hsp90 either for efficient replication or proper intracellular transfer. A recent proteomics study revealed that Hsp90 is incorporated into the budded virions (BVs) of baculovirus, we therefore investigated the role of Hsp90 in the life cycle of baculovirus. By using Hsp90 inhibitor geldanamycin (GA) and RNA interfering, the levels of viral DNA replication, infectious BV production, as well as ODV and polyhedra morphogenesis of baculoviruses were significantly reduced in AcMNPV infected cells. Further studies demonstrated that GA inhibited the expressions of certain viral proteins at transcriptional levels. The nuclear imports of several nucleocapsid- and ODV envelope proteins were also hindered by GA. Interestingly, when the function of Hsp90 was disturbed by GA, virus-triggered nuclear F-actin network essential for assembly of progeny AcMNPV was absent. Taken together, our data suggest that Hsp90 regulates baculovirus replication and morphogenesis from at least three different aspects: 1) promoting the expression of viral proteins; 2) facilitating the intracellular trafficking of viral structural proteins; 3) participating in the nuclear polymerization of host actin which is required for progeny baculovirus production.

Contributed paper. Thursday, 14:30. **244**

Development and immunity-related microRNAs of the lepidopteran model host _Galleria mellonella_

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MicroRNAs (miRNAs) are small non-coding RNAs which have been recognized as key elements in the regulation of protein synthesis at the post-transcriptional level. Our knowledge about their function in regulating complex physiological processes is limited, but rapidly expanding. The larvae of the greater wax moth *Galleria mellonella* have emerged as a powerful and surrogate model hosts for pathogens capable of infecting insects or humans. Complementary to our previously published comprehensive *G. mellonella* transcriptome, here we screened development and immunity-related miRNAs in order to further advance the suitability of this model host. To screen for miRNAs that are differentially expressed in *G. mellonella* either during metamorphosis or upon natural infection with entomopathogenic bacteria or fungi we designed

a microarray spotted with probes of more than two thousand miRNA sequences known from insects. Relative to untreated last instar larvae which were used as a reference, we determined numerous miRNAs to be expressed in prepupae (1037), pupae (981) or pathogen infected last instar larvae (965). Taking advantage of our transcriptomic data base, we were able to identify potential 3' UTRs for determining miRNA-mRNA duplexes by considering both base pair complementarity and minimum free energy (MFE) hybridization. We confirmed the co-expression of selected miRNAs such as miR-71, miR-263a, miR-236b, and their predicted target mRNAs in *G. mellonella* by RTPCR. This is the first study addressing the identification of miRNAs which are predicted to regulate genes that are expressed during metamorphosis or in response to infection of the lepidopteran model host *G. mellonella*.

Contributed paper. Thursday, 14:45. **245**

The *sf122* gene of *Spodoptera frugiperda* nucleopolyhedrovirus modulates key aspects of insect-to-insect transmission and post mortem host liquefaction

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The *sf122* gene present in the longest genotype (SfMNPV-B) of the Nicaraguan isolate of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) was previously identified as undergoing positive selection. A recombinant virus (Sf122null), lacking *sf122,* was generated by homologous recombination from a bacmid comprising SfMNPV-B. Transcriptional analysis revealed that *sf122* is a late gene. Sf122null DNA was two-fold less infective when injected into *S. frugiperda* larvae and occlusion bodies (OBs) of the deletion recombinant were 15-fold less pathogenic (in terms of 50% lethal concentration), speed-of-kill was slower by 20 hours and OB production was reduced 3-fold, compared to the parental virus. The infectious titre of occlusion derived virions (ODVs) of Sf122null was reduced by >100-fold compared to that the parental or *sf122*-repaired viruses. OBs from each virus did not differ significantly in DNA content or gross morphology. Larvae that died from Sf122null infection did not show liquefaction. Similarly, SfMNPV isolates from the United States and Colombia, containing the shorter variant of the protein, only produced partial larvae liquefaction post mortem. Finally, expression of the *chitinase* and *cathepsin* genes was significantly reduced in larvae infected with the Sf122null virus. We conclude that positive selection on the *sf122* gene is most likely related to its marked role in modulating larval liquefaction and virus transmission.

Contributed paper. Thursday, 15:00. **246**

Effect of a Viral Encoded Protein Kinase on Gene Expression in Amsacta moorei Entomopoxvirus Infected Cells

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Insect-born entomopoxviruses (EPVs, Family: *Poxviridae*) are potentially significant biotechnological tools. In comparison to some other insect viruses, the function of relatively few EPV gene has been characterized. In this study, a serine/threonine (Ser/Thr; ORF AMV197) protein kinase gene of the Amsacta moorei entomopoxvirus (AMEV, type species of *Betaentomopoxvirus*) was characterized in terms of regulation of expression relative to some other AMEV genes. A recombinant virus (AmΔPK/*gfp*) was constructed by deleting ORF197 from AMEV genome via homologous recombination. Transcription of wild type virus and recombinant virus genes was compared by whole-genome gene expression microarray. The results showed that the expression levels of 126 genes representing 55.7% of all the viral genes were impacted significantly in the deletion mutant virus. Of these, 88 (69.84 %) transcripts were up-regulated and 38 (30.15 %) were down-regulated. Specifically, transcripts responsible for DNA repair, replication, nucleotide metabolism, and transcription and RNA modification were up-regulated in AmΔPK/*gfp*-infected cells. The results of this study indicate that the product of AMV197 may have significant effects on the assembly and/or infectivity processes of progeny viruses. However, more detail experiments are necessary to identify the exact role of this gene in AMEV replication.

Contributed paper. Thursday, 15:15. **247**

FP25K acts as a negative regulator in the infectivity improvement of AcMNPV Budded viruses

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Baculoviruses can produce two phenotype virions in the replication cycle, the budded virus (BV) and the occlusion-derived virus (ODV). The regulation of forming these two phenotypes virions is an important process in infection, but the mechanism is still unclear. The *fp25k* gene was reported to be responsible for the regulation of BV/ODV formation. The gene mutation results in a decreased number of normal ODV and an increased production of BV. In this study, we unraveled the mechanism of improved infectivity of *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) BVs by *fp25k* deletion. The investigation of BV titer, copy number of BV genome and electro-microscopy observation indicated that the increase of BVs titer of the *fp25k* knock out recombinant is a result of higher infectivity of virions but not the amount of BVs. The identification of protein associated to the virions showed that more BV envelope protein was incorporated into the gene knock out recombinant BVs. However, the infectivity of BVs was confirmed be not increased when GP64 was over expressed in our study. From the transfection and transformation of BV genome DNA into insect cells and *Escherichia*.coli, the results suggested that better integrity genome DNA was packaged in the *fp25k* knock out recombinant BVs. Our study proposed that FP25K is a multifunctional protein in baculovirus life cycle. The virus genome with better integrity might be the major reason of infectivity enhancement and FP25K acts as a negative regulator in this process.

Contributed paper. Thursday, **248**

The leucines in the transmembrane domain of *Autographa californica nucleopolyhedrovirus* Ac76 are important for intranuclear microvesicle formation

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Our previous study has shown that the *Autographa californica nucleopolyhedrovirus* (AcMNPV) *ac76* gene is essential for both budded virion (BV) and occlusion-derived virion (ODV) development. However, the exact role by which *ac76* affects virion morphogenesis remains unknown. In this report, the oligomerization status of Ac76 was investigated and its critical amino acids for intranuclear microvesicle formation were identified to further understand the functional role of Ac76 in virion morphogenesis. Ac76 contains an α-helical transmembrane domain (TM), and phase separation showed that it is an integral membrane protein. In AcMNPV-infected cells, Ac76 was detected as a stable dimer that was resistant to SDS and thermal denaturation, and only a trace amount of monomer was detected. A co-immunoprecipitation assay demonstrated the dimerization of Ac76 by high-affinity self-association. Covalent cross-linking results showed that higher-order oligomers of trimer, tetramer, hexamer and octamer as well as the stable dimer were detected in virus-infected cells. Bioinformatic analysis suggested that the leucine- and isoleucine-rich sequence in the TM helix of Ac76 likely forms a leucine/isoleucine zipper to mediate the helix-helix interaction of Ac76 with itself. A recombinant virus in which L^{26}, L^{29} and L^{33} in the TM of Ac76 were all substituted with alanines was constructed. Analysis of the mutant revealed that the leucines in the TM of Ac76 are important for infectious BV production and normal-appearing intranuclear microvesicle formation.

Contributed paper. Thursday, 15:30. **249**

High-throughput purification of dsRNA against sacbrood virus disease in honey bees *Apis cerana* (Hymenoptera: Apidae)

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The importance of honey bees to the world economy does not hang on bee products, but pollination of 80% food crops. However, like other animals, honeybee is inevitably subject to infection by a wide variety of pathogens that are responsible for significant colony losses. Sacbrood virus (SBV) is a serious hazard disease to honey bees (*Apis cerana*). Relying heavily on chemical agent for the control of this disease, problems of resistance and pollution are perplexing beekeeping. Therefore, beekeeping calls for environmentally friendly technology of disease management, especially the antiviral bee breeding. Using RNA interference technology is a cost-effective approach for disease bio-control. To address this issue, large-scale and pure dsRNA is in great need. A length of 699 bps *Vp1* gene of SBV was selected to be expressed with L4440 plasmid in *Escherichia coli* HT115 (DE3). After ultrasonic disruption and ethanol precipitation, *Vp1*-dsRNA molecules were purified with anion exchange chromatography utilizing convective interaction media (CIM) monolithic columns. RNAi was performed to prevent bees from SBV under laboratory conditions. Comparing with bees without dsRNA, *Vp1*-dsRNA prevented 49% to 75% larval mortality of *A. cerana* from SBV infection. The result may provide a model in large-scale use of RNAi for SBV control.

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