suggest that *Wolbachia* may interfere with the establishment and transmission of this important DNA virus (SGHV), which represents a major hurdle for the application of SIT strategies for the control of tsetse flies and trypanosomosis in sub-Saharan Africa.

Contributed paper. Thursday, 8:15. 216-STU

Mechanisms of tree-top disease induced by the specialist baculovirus SeMNPV

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Many parasites alter host behavior to enhance their transmission or survival. An intriguing example is the altered behavior of insect larvae infected by a baculovirus, e.g. their movement to elevated positions. This phenomenon (tree top disease or Wipfelkrankheit) is already known for over a century. However, the underlying mechanisms leading to this behavioral adaptation are still largely enigmatic. Here we studied tree-top disease induced by the baculovirus Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) in S. exigua larvae. We show that infected S. exigua caterpillars all climb to elevated positions prior to death. Furthermore, we investigate the role of the ecdysteroid UDPglucosyl transferase (egt) gene from SeMNPV in tree-top disease. This gene is known to be important in tree-top disease in another baculovirus-host system, although the mechanism by which it exerts this effect is unknown. We hypothesize that the SeMNPV egt gene may directly trigger tree-top disease or induce this phenomenon indirectly by prolonging the larval time to death.

Contributed paper. Thursday, 8:30. 217

Temporal proteomics to study virus infection and function in the host cell

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Invertebrate iridescent virus 6 (IIV-6) is a nucleocytoplasmic virus with a 212 kb-long linear double-stranded DNA genome that encodes 215 putative open reading frames. The IIV-6 virion proteome consists of at least 54 virally-encoded proteins. One of our previous findings showed that most of these proteins are encoded by genes from the early transcriptional class. This indicates that these structural proteins may not only function in the formation of the virion, but also in the initial stage of viral infection. In the current study, we followed the protein expression profile of IIV-6 over time in Drosophila S2 cells by label-free quantitation using nanoLC-FTMS. A total of 95 viral encoded proteins were detected in infected cells, of which 37 are virion proteins. The expressed IIV-6 virion proteins could be categorized into three main clusters based on their expression profiles. These clusters were: 1) proteins with stably low or 2) exponentially increased expression levels during infection, and 3) proteins that were initially highly abundant, and then showed slightly reduced levels after 48 hours (h) post infection (p.i.). The study supported that temporal expression patterns did not share direct correlation with protein expression classes

phenomena, suggesting that both proteomic and transcriptomic approaches will be required to obtain a detailed understanding of the viral expressomics (infectome). Here, we provide novel information on the kinetics of virion and infected cell-specific protein levels that assists in understanding gene regulation in this lesser known DNA virus model.

Contributed paper. Thursday, 8:45. 218

Characterization of an atypical fast-killing ascovirus: Spodoptera frigiperda ascovirus 1d (SfAV-1d) Eiko Arai¹; Shiori Sagawa¹; Yasumasa Saito¹; Xiao-Wen Cheng²; Dennis Bideshi^{3,4}; Maki Inoue¹; Yasuhisa Kunimi¹; Brian Federici³; <u>Madoka Nakai¹</u> ¹Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; ²Department of Microbiology, 32 Pearson Hall, Miami University, Oxford, Ohio 45056, USA; ³Department of Entomology, University of California, Riverside, Riverside, California 92521, USA; ⁴California Baptist University, Riverside California 92405 Address for Correspondence: madoka@cc.tuat.ac.jp

Ascoviruses (AVs) are large double-stranded DNA viruses that attack lepidopterans, mainly noctuid larvae. One of the unusual features of AVs is their mode of transmission via parasitoid wasps. AVs are poorly infectious per os compared to other insect viruses such as baculoviruses and cypoviruses. Additionally, AV infection results in production of a characteristic milky-white hemolymph due to accumulation of virion-containing vesicles produced by a modified apoptotic response in infected cells. Virtually all ascoviruses cause a chronic disease wherein larvae survive for as long as 28 days after infection, which enables an extended period of transmission among larvae by wasps. Here, we report characterization of Spodoptera frugiperda ascovirus 1d (SfAV-1d) isolated from a S. frugiperda larva. SfAV-1d killed S. litura 4th instar larvae within 3 days when compared to another AV (SfAV-N), which took as long as 23 days to kill larvae. Larvae infected with SfAV-1d contained the characteristic white hemolymph. Electron microscopy revealed that both SfAV-1d and SfAV-N infected the fat body but not the tracheal matrix or other tissues. Interestingly, despite the difference in the rate at which SfAV-1d and SfAV-N killed larvae, there was no apparent difference in the kinetics of viral DNA replication. The primary difference between these two isolates was that SfAV-1d formed and accumulated virion-containing vesicles in the hemolymph much more rapidly than SfAV-N. Our future studies will focus on characterizing the genetic differences between these viruses to identify determinants that influence their pathobiology, particularly as it relates to rate of kill.

Contributed paper. Thursday, 9:00. 219-STU

Two nucleopolyhedroviruses isolated from the genus Adoxophyes inhibit juvenile hormone (JH) esterase activity but not JH epoxide hydrolase activity

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Insect metamorphosis is predominantly regulated by two hormones, juvenile hormone (JH) and ecdysone. During the final instar, a dramatic decrease in JH titer is required for the induction of pupation. JH is metabolized by two enzymes, JH esterase (JHE) and JH epoxide hydrolase (JHEH). Adoxophyes honmai (Lepidoptera: Tortricidae) is susceptible to two nucleopolyhedroviruses (NPVs), A. honmai NPV (AdhoNPV) and A. orana NPV (AdorNPV), which are genetically closely related but differ in killing speed. AdhoNPV kills the host only in the final instar, whereas AdorNPV kills more quickly (5 to 8 days). When 4th instars of A. honmai are orally inoculated at $>LC_{95}$ (1.0 x 10⁸ OBs/ml), AdhoNPV and AdorNPV prevent pupation and kill the host in 10 and 8 days, respectively. In contrast, mock-inoculated larvae pupate in 7 days. Baculoviruses are known to prevent pupation through endocrynological regulation. Here, we monitored both JHE and JHEH activities in AdhoNPV-, AdorNPV-, and mockinfected larva of A.honmai. Mock-infected larvae showed increased JHE activity in the hemolymph and fat body during the final instar, with the highest activity found on the 3rd day of the 5th instar. Both AdhoNPV- and AdorNPV-infected larvae did not show JHE activation. On the other hand, JHEH activity in fat body was constant and no differences were found between treatments. Our data suggest that AdhoNPV and AdorNPV prevent pupation by specifically down-regulating JHE but have no effect on JHEH activity. Our data also suggest that JH titers remain relatively high during the final instar of baculovirus infection.

Contributed paper. Thursday, 9:15. 220

Mechanism underlying virus-induced hyperactive behavior: Substrate identification of the baculovirus protein tyrosine phosphatase

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Many parasites alter the behavior of their host to maximize their transmission and survival. However, the underlying mechanisms are largely unknown. Baculoviruses manipulate the behavior of their caterpillar hosts by inducing hyperactivity and climbing behavior. Previous work demonstrated that a protein tyrosine phosphatase (PTP) encoded by the californica baculovirus Autographa multiple nucleopolyhedrovirus (AcMNPV) was involved in the induction of hyperactive behavior in Spodoptera exigua larvae. This finding prompted us to investigate which viral and/or host proteins interact with the baculovirus PTP enzyme and might be involved in altered host behavior. Using affinity-tag purification of a substrate-trapping mutant of AcMNPV PTP incubated with extracts of infected cells followed by proteomic analysis of the trapped protein, we identified six viral and six host proteins that co-purified with PTP. Several of these proteins are known to be important in cellular signaling and in behavior in other insects/organisms, and are therefore potentially involved in PTP-mediated hyperactivity of infected larvae. For one of these identified host proteins, the 14-3-3 ζ protein, RNA expression levels were significantly higher for AcMNPV wild type-infected larvae as compared to AcMNPV Δptp -infected larvae, indicating that 14-3-3 ζ expression levels are dependent on the presence of the baculovirus ptp gene. The 14-3-3 ζ protein is known to be important for the synthesis of serotonin and dopamine, which are neurotransmitters that play important roles in many behavioral pathways. It is hypothesized that baculovirus *ptp* targets 14-3-3 ζ at both the RNA and protein level, which consequently leads to baculovirus-induced hyperactivity.

Contributed paper. Thursday, 9:30. 221-STU

The genome of a baculovirus isolated from *Lonomia* obliqua (Lepidoptera:Saturniidae) reveals a new transcription terminator factor possible acquired from the host

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Lonomia obliqua (Lepidoptera: Saturniidae) is a poisonous larvae of medical importance due to the severity of accidents occurred in Brazil caused by the contact of these larvae with the human skin. The possibility of controlling these populations is being evaluated by using pathogens such as a nucleopolyhedrovirus isolated from L. obligua. In this work, we have sequenced the genome of the baculovirus LoobMNPV and analyzed its genomic composition and evolutionary history. The genome is 120.022 bp long, comprising 135 putative ORFs. Furthermore, in an evolutionary context, based on analysis that include the core gene from 93 sequenced baculovirus. LoobMNPV fell into a basal position related to the Alphabaculovirus group I (lepidopteran-infective NPV). Interestingly, one ORF showed significant identity (e-value equals to 3e10⁻¹¹) to a eukaryotic transcription terminator factor (TTF2) from the lepidoptera Danaus plexippus (GenBank: EHJ68439.1). On the other hand, when restricting this search only to baculoviruses, this ORF also demonstrated identity (e-value of 1e10⁻⁶) to the Global Transactivator (GTA) gene from Antheraea pernyi nucleopolyhedrovirus Phylogenetic (Genbank:YP_611073.1). analysis were performed with the TTF2 gene from various organisms, as well as with the GTA from baculovirus. These results indicated two hypothesis: (i) this gene may have been independently acquired from the host through horizontal transfer, acting as an inhibitor of the host's transcriptional machinery in order to benefit viral translation; (ii) or it is a divergent variation of the GTA gene that has undergone positive selection.

Contributed paper. Thursday, 9:45. 222

The essential baculovirus protein VP1054 is a hijacked cellular PURα,

a nucleic-acid-binding protein specific for GGN repeats Martin Marek¹, Christophe Romier¹, Lionel Galibert², Otto-Wilhelm Merten², and <u>Monique M. van Oers</u>³ ^{1.2.3}Biologie Structurale Intégrative, Institut de Génétique et

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The baculovirus VP1054 protein is a structural component of both budded virus (BV) and occlusion-derived virus (ODV), but its exact role in virion morphogenesis is poorly defined. We reveal sequence and functional similarity between the baculovirus protein VP1054 and the cellular purine-rich element binding protein PUR-alpha (PURa). The data strongly suggest that gene transfer has occurred from a host to an ancestral baculovirus. Deletion of the AcMNPV vp1054 gene completely prevented viral cell-to-cell spread. Electron microscopy data showed that assembly of progeny nucleocapsids was dramatically reduced in the absence of VP1054. More precisely, VP1054 is required for proper viral DNA encapsidation, as deduced from the formation of numerous electron-lucent capsid-like tubules. Complementary searching identified the presence of genetic elements composed of repeated GGN trinucleotide motifs in baculovirus