Contributed paper. Tuesday, 9:45. **78**

Improvement of UV-resistance of Baculovirus by displaying the Nano-material binding peptides on the Polyhedron Envelope

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Baculoviruses are sensitive to UV radiation and this characteristic causes the control efficacy of viral insecticides unsteady in the fileds. The polyhedron envelope of baculoviruses, which is composed of carbohydrate and phosphorylated protein (PEP), is the first barrier against the disadvantageous environment. We found that orthologs of *Autographa colifornica multiple nucleopolyhedrovirus* (AcMNPV) PEP, such as *Helicoverpa armigera nucleopoly-hedrovirus* PEP, *Cydia pomonella granulovirus* Cp20 or Cp22 might not repair the absence of polyhedron envelope in the *pep*-knocked-out AcMNPV construct. The C-terminal (168~252aa) of AcMNPV PEP might deliever GFP to be expressed on the surface of polyhedron. Consequently, we had constructed the AcMNPV recombinants in which the C-terminal of PEP was fused with the peptides which might specifically bind melanin or nano-scale ZnO. These results may lay a foundation for developing intensive UV-resistant viral insecticides.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

BACTERIA 2

Contributed paper. Tuesday, 8:00. **79**

***Yersina entomophaga* MH96 (Enterobacteriaceae) BC subcomplex of the Yen-Tc ABC toxin is able to induce toxicity independent of the A subcomplex**

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A novel gram-negative, rod-shaped, non-spore-forming bacterium, *Yersinia entomophaga* MH96 (Enterobacteriaceae), was isolated from diseased larvae of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). *Y. entomophaga* produces a proteinaceous toxin complex (Yen-Tc) that is responsible for mortality in a range of insect species, mainly within the Coleoptera and Lepidoptera. The Yen-Tc is made up of two chitinase subunits (Chi1 and 2) and five Yen subunits (A1, A2, B, C1, and C2). The TcA, B, and C subunits are related to members of the Toxin complex (Tc) toxin family, with orthologs identified from several other bacterial species including *Serratia entomophila* and *Photorhabdus luminescens*. Characterization of Yen-Tc pathology has revealed a progressive deterioration of the midgut epithelium of susceptible insects. Although the specific mechanism of Yen-Tc remains unknown, cellular and molecular work has begun shedding light on how the Tc family functions. The current model proposes that the TcA component binds to the cell surface and forms a pH-triggered channel that allows translocation of the TcBC subcomplex into the cytoplasm. Once in the cytoplasm the carboxy-terminus of the TcC subunit dissociates and becomes active, which causes toxicity in both insect and mammalian cells. A major component of this model is the requirement of an intact toxin complex in allowing TcBC to be transported into the cell. Based on our investigations of Yen-Tc, the YenBC subcomplex and the YenC subunit do not necessarily require full complex assembly to trigger cell toxicity. We will present and discuss our findings in relation to the current model.

Contributed paper. Tuesday, 8:15. **80**

Interaction of *Bacillus thuringiensis* Cry1Ab toxin with Mucus-rich structures

Diego Segond[1,2], Agnès Rejasse[1], Christophe Buisson[1], Shuyuan Guo[1,3], Karine Adel-Patient[2,4] , Hervé Bernard[2,4], Didier Lereclus[1], Christina Nielsen-LeRoux[1],
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Bacillus thuringiensis larvicidal Cry toxins are currently known for their strong host specificity; which is mainly due the presence of specific toxin binding sites on midguts of susceptible insect larvae. Meanwhile Cry toxins can also bind to compounds in the peritrophic matrix (PM) of several insects (*Rees *et al.* 2009; Valaitis and Podgwaite 2013). In *G. mellonella* infected with toxin alone, we observed structural modification of the peritrophic matrix but no evidence for the biochemical explanation for this modification is found so far. Knowing that "mucus" is along with chitin the main components of PM and that mucus is commonly found in several organisms, we aim to investigate the capacity of Cry1Ab to bind to several mucus rich structures. Indeed, our hypothesis is that the heavily glycosylated proteins (peritrophins and mucins) and proteoglycans shared by both vertebrate and invertebrate mucus may bind Cry toxins, therefore questioning on the "specificity" of these toxins used in GMO crops. Using, commercial pork stomach mucins, mice intestinal mucus, vertebrate cell-culture mucus and PM and peritrophins from *G. mellonella,* we then deeply analyzed Cry1Ab-mucus interactions. The presentation will deal with results from far western blot studies, ELISA binding experiments, inhibition ELISA with sugars, lectins or anti-Cry1Ab monoclonal antibodies. Identification of the interacting structure by LC/Ms/Ms analysis and resulting toxicity using insect and cellular models will be also shown.

*J Invertebr Pathol. 2009 Mar; 100(3):139-46; J Invertebr Pathol. 2013 Jan; 112(1):1-8.

Contributed paper. Tuesday, 8:30. **81-STU**

Pore formation helping ability and binding affinity of BmABCC2 and BtR175 against Cry1A toxins.

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By *in vitro* toxicity assay using Sf9/Baculovirus expression system, we previously provided a novel evidence that *Bombyx*

mori ABC transporter C2 (BmABCC2) functions as a receptor for Cry1A toxins. We also demonstrated that BmABCC2 can confer approximately 10-1000 times higher suscetibility to the cells than cadherin-like receptor (BtR175) and BmABCC2 and BtR175 co-expression exerts synergistic effect in susceptibility confering ability. This synergistic effect sugested that these two receptors have different roles in the mode of action of Cry1A toxins in Sf9. Thus, we addressed to find the difference in the roles of the two receptors. First, we evaluated pore formation helping ability of the receptors using xenopus oocyte expression system and the two-microelectrode voltage clamp technique. When Cry1Aa or Cry1Ab toxin was administrated to BmABCC2 expressing oocytes, current continuasly increased during toxin incubation, indicating that pores were continuasly assembled on the cell mambrane. However, when BtR175 expressing oocytes were administrated with toxins current increment speed was lower than in BmABCC2 expressing oocytes, indicating that BtR175 has lower function than BmABCC2 in pore formation helping. In contrast, BmABCC2 and BtR175 co-expressing oocytes showed at least 4 times higher current increment speed than BmABCC2 single expressing oocytes. This indicates that synersism occurs at least in part in the pore formation process, although synergistic effect is very low in comparison to that seen in Sf9 expression sysytem. We also compared the binding affinity to Cry1A toxins of BmABCC2 and BtR175 using Biacore systems. We will discuss these results, too.

Contributed paper. Tuesday, 9:00. **82**

A necessary step in the mode of action of the Cry8 toxin: the elimination of DNA from the Cry toxin-DNA complex

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Several crystal (Cry) proteins are known to occur as DNA-protein complexes. However, the role of the DNA associated with the activated toxin in the mechanism of action of the Cry toxin has long been ignored. Here, we focused on the DNA-activated Cry toxin complex. Both forms of the Cry8Ca2 and Cry8Ea1 toxins, i.e., with or without bound DNA, were separately obtained. Size-exclusion chromatography analysis indicated that the Cry8Ca2 toxin-DNA complex has a tight or compact structure. The Cry8 toxin-DNA complex is more likely to move toward the air/water interface and is more hydrophobic than the toxin without DNA. Competitive binding assays indicated that the Cry8Ca2 and Cry8Ea1 toxins without DNA specifically bind to the midgut of *Anomala corpulenta* and *Holotrichia parallela* larvae, respectively. In contrast, the association of DNA with each toxin might result in the nonspecific recognition of the Cry toxin and its target receptor in the insect midgut. The association of the DNA fragment with the Cry8 toxin was shown to protect the Cry protein from digestion by proteases. Based on our results, we propose an additional step in the mechanism of action of the Cry8 toxin and elucidate the function of the associated DNA as well as the importance of the removal of this DNA for the insecticidal activity of the toxin.

Contributed paper. Tuesday, 9:15. **83 STU**

How does the *Bt* Cry41Aa toxin kill human cancer cells?

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In this study the cytocidal activity associated with the Cry41Aa human cancer cell-active toxin of *Bacillus thuringiensis* (*Bt*), also known as Parasporin-3, was characterized. We investigated the effects of recombinant Cry41Aa on the human hepatic cancer cell line HepG2 to elucidate its mode of action. Cry41Aa shares structural homology with commercially used insecticidal toxins. The fact that some *Bt* toxins are able to kill mammalian cells may threaten the use of *Bt* toxin-based pesticides in the future. Moreover the preferential and narrow cytocidal activity of Cry41Aa has potential for anticancer drug design. Significant uptake of fluorescent dye was observed in susceptible cells as little as 10 minutes post administration, suggesting rapid membrane damage. Microscopic observation revealed cellular and nuclear swelling induced within the first hour of treatment. The activation of apoptosis effectors Caspase 3/7 was not observed within 24 hours, although phosphorylation of p38 MAP kinase was. Our results suggest that Cry41Aa, like its insecticidal homologues - but unlike some other Parasporins, is a pore-forming toxin that rapidly increases membrane permeability in the target cell. Research is on-going to identify whether a specific receptor is present on the surface of susceptible cells.

Contributed paper. Tuesday, 9:30. **84 STU**

Which regions of the *Bt* Cry41Aa toxin are responsible for its activity against human cancer cells?

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The *Bacillus thuringiensis* human cancer cell-active Cry41Aa toxin (Parasporin3) contains the five conserved sequence blocks found in many insecticidal toxins and is believed to possess the same three domain fold. However, Cry41Aa is predicted to have an extra loop in its domain II as well as an additional "ricin" domain at its C-terminus. Deletion of the "ricin" domain resulted in a stable protein with a toxicity to HepG2 cells not significantly different to the non-modified toxin. Several deletions of the loop region all resulted in an unstable protein that could not be further analyzed. Various bioinformatic procedures were used to identify the loops at the apex of domain II that have previously been implicated in receptor binding in the insecticidal Cry toxins. A range of mutations in the putative loop 1 were made but none affected toxicity to HepG2. In loop 3 the presence of an aromatic residue at position 509 was found to be important for toxicity. In an attempt to further dissect which regions are important for toxicity hybrids have been made between insecticidal and cancer cell-active toxins. Our data to date suggest that Cry41Aa has a mechanism of action similar to the three-domain insecticidal Cry toxins.

Contributed paper. Tuesday, 9:45. **85**

Parasporin PS1Aa2 induces ionic channels in lipid bilayer membranes and calcium oscillations in sensitive cells

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Parasporins are *Bacillus thuringiensis* Cry toxins that are active against tumor cells. Like many Cry toxins, parasporin

PS1Aa2 (Cry31Aa2) formed pores in artificial membranes. These pores had several levels of conductance; the most frequently observed in 150 mM KCl solutions were of 11, 16 and 21 pS. Microspectrofluorometric experiments with the Fura-2 probe showed that the presence of PS1Aa2 can induce changes in intracellular calcium levels, most often in the form of calcium oscillations and sometimes as sustained increases. Such responses were observed in the presence and absence of extracellular calcium, with the tumor cell lines HeLa and HepG2, and with the non-tumorous cell line HEK 293. Calcium oscillations have not been described previously for Cry toxins even though some studies have shown that calcium appears to be involved in their mode of action. Our experiments required the use of much higher concentrations of toxin than suggested from the published cytotoxicity results. Despite the presence of fragments previously identified as active, its low efficacy appears to be related to the presence of DNA in the preparations causing the protein to precipitate. Future work aimed at elucidating the origin of these calcium oscillations and their role in toxicity will be greatly facilitated by an improvement in the method of preparation of this toxin.

Contributed paper, 10:00. **86-STU**

Caenorhabditis elegans – _Bacillus thuringiensis_ interactions: new insights into mechanisms of host resistance and pathogen virulence

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Genetically tractable model nematode *Caenorhabditis elegans* has been successfully used in the host-pathogen interaction studies and helped to uncover conserved virulence factors of clinically relevant pathogens. At the same time interactions of this nematode with its natural pathogens are poorly investigated. Bacteria from the genus *Bacillus* are among potential natural pathogens of the nematodes. Therefore, previously we isolated 768 *Bacillus* strains and tested them for the virulence to nematodes. Although only 3% of tested *Bacillus* strains were pathogenic, one strain called *B. thuringiensis* DB27 exhibited extreme virulence to *C. elegans*. Currently we are trying to tackle both sides of this host-pathogen equation and aiming to identify virulence factors of *B. thuringiensis* DB27 as well as *C. elegans* defense mechanisms. First, combining plasmid-curing and genome sequencing, we discovered that novel nematicidal Cry21 toxins with synergistic activity are the main nematicidal factors of DB27. We expressed these novel toxins in *E. coli* and confirmed their activity against *C. elegans*. Importantly, these toxins are also active against other free-living and animal parasitic nematodes, suggesting their potential application against parasitic nematodes. Our parallel work on the host side led to the discovery of *C. elegans* novel innate immunity pathway involved in the defense against pathogens. Specifically, we identified novel function for Dicer in *C. elegans* antibacterial innate immunity and showed that this function is largely associated with microRNA processing. Taken together, our reciprocal studies uncovered a previously unknown role for DCR-1/Dicer in *C. elegans* antibacterial immunity as well as identified novel nematicidal toxins.

SYMPOSIUM 4 (Viruses) Tuesday, 10:30-12:30

Small non-coding RNAs as regulators of insect host-virus

Symposium. Tuesday, 10:30. **87**

Role of cellular and virus-encoded microRNAs in insect host-virus interactions

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MicroRNAs (miRNAs) are small non-coding RNAs of ~22 nucleotides which play significant roles in gene regulation at transcriptional as well as post-transcriptional levels. They are produced in almost all eukaryotes and also encoded by some viruses. Besides cellular miRNAs that may participate in anti-viral responses following infection, virus-encoded miRNAs may target host genes to interfere with host survival, proliferation and immunity. Furthermore, virus-encoded miRNAs may regulate replication of virus to avoid over replication and quick demise of the host or facilitate virus entry into persistent infection. The interaction may become more complicated in the presence of third parties, such as microbiota and endosymbionts, that in turn may affect the host's miRNA profile and indirectly disturb virus replication. In the presentation, the role of miRNAs in mosquito-arbovirus interactions with a reference to the effect of Wolbachia as an endosymbiont on the interactions will be discussed.

Symposium. Tuesday, 11:00. **88**

Sensing viral RNA in _Drosophila melanogaster_

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RNA interference plays a central role in antiviral innate immunity in flies. Indeed, flies mutant for the three key components of the small interfering (si)RNA pathway, namely Dicer-2, R2D2 and Argonaute (AGO) 2 are highly sensitive to a wide range of viruses (1). Dicer-2 produces virus derived-siRNAs from viral RNAs throughout its RNaseIII activity. The Dicer-2/R2D2 heterodimer then loads these siRNAs onto AGO2 in the RNA-induced silencing complex, RISC. The RISC complex is then able to target viral RNAs, thus impairing the ability of the virus to successfully replicate. Although *in vitro* and *in vivo* experiments clearly indicate that Dicer-2 can process long double stranded RNA, the exact nature of the viral RNAs sensed *in vivo* in infected cells remains mysterious. We are interested in understanding how Dicer-2 senses viral RNAs, with a particular focus on the contribution of the N-terminal DExD/C helicase domain, which is conserved in mammalian RIG-I like receptors. Indeed, *in vitro* experiments have revealed a critical role of this domain in both processivity of the enzyme and discrimination of the extremities of the template RNA (1,2). To address this question, we take advantage of a combination of approaches including *Drosophila* genetics, next-generation sequencing technologies and bioinformatics analysis.

(1) Kemp *et al.*, J. Immunol. 2013
(2) Cenik *et al.*, Mol Cell. 2011