CSE62C

Report on overseas travel

Karl H.J. Gordon, CSIRO Division of Entomology

Summary

The major aims of this trip were to attend three international conferences in areas of entomology, insect pathology and virology relevant to the long-term biological control of major cotton pests like *Helicoverpa armigera*:

1. Xth International Congress of Virology.

2. XXth International Congress of Entomology.

3. XXIXth Annual Meeting of the Society for Invertebrate Pathology (SIP).

A further major aim was to attend a Management Committee Meeting, hosted by Zeneca Agrochemicals/Seeds (UK), to review progress on the collaborative *Helicoverpa armigera* stunt virus (HaSV) project aimed at exploiting HaSV genes for control of heliothine pests on cotton. This work is partly funded by the CRDC under CSE55C and described in reports concerning that project.

Work reported at the three conferences covered many key areas of insect molecular biology relevant to our use of insect viruses for pest control. They provided valuable and up to date insights into recent research into animal, plant and insect viruses and into the host responses observed upon virus infection. These responses are important both in determining the limits to a virus infection - can the host contain the pathogen, or does the pathogen win the battle to grow? - and how does the virus cause disease? Our understanding of the disease process is growing rapidly due to studies on the interaction between viruses and the host cells within which they grow, and of the functions of both virus genes and of host genes required for virus growth.

The genetic engineering of viruses as either vectors for foreign genes or in order to alter their pathological properties was another subject covered at these meetings. These reports gave insights into strategies for the engineering of DNA viruses (e.g. the baculoviruses) or RNA viruses (resembling HaSV), as well as for the production of viruses in cells other than those of their normal hosts by a variety of recombinant means. The latter makes feasible the economic production of viruses like HaSV which are otherwise difficult or impossible to make outside their natural hosts.

Reports on our work with HaSV were presented at all three meetings. These allowed critical discussion of the HaSV work with international experts in relevant disciplines and attracted the attention of other workers interested in control of insect pests. A significant outcome was to raise the interest of other scientists in studying the response of major, but little studied pests like the heliothis complex to pathogens, especially viral pathogens, with the aim of developing future generations of safe and specific biological control agents.

Xth INTERNATIONAL CONGRESS OF VIROLOGY (ICV)

This conference provided a valuable opportunity to learn of recent work on a wide variety of animal and plant viruses. Reports on animal virus work generally concerned the intecation between virus and host at all levels, from the intercellular to the whole organism. Reports on plant viruses covered the interaction between virus and host, transmission by insects, the engineering of resistant plants and the use of viruses in antigen production. Reports on insect viruses generally concerned the engineering of baculoviruses for pest control.

Major issues:

(1) Viruses and apoptosis

Programmed cell death, or apoptosis, is increasingly recognized as a common response of animal cells to virus infection. Work with baculoviruses has implicated it as a primitive insect immune response to viruses found in insects, although there may be other, cellular, responses too. Our recent finding that HaSV induces apoptosis in larval midgut cells has opened up new approaches for pest control based on the manipulation of this cellular response to external agents. Unlike baculoviruses, HaSV appears not to carry any genes capable of preventing or delaying apoptosis in infected cells, raising the question of how the stunt virus is able to overcome what appears to be a defence or immune response to virus infection. (Timely death of virus-infected cells reduces the yield of virus from that cell and its ability to spread in the animal.)

Large DNA viruses like the adenoviruses carry a number of genes whose products manipulate the cellular apoptotic response (E. White, Rutgers Unniversity; W. Wold, St Louis University). Several of these viral genes serve to block the apoptosis pathway at different points, and thereby allow virus replication over a longer period than would otherwise occur. A further adenovirus gene, encoding the Death Protein, is switched on very late in the infection cycle and kills the cell by a non-apoptotic mechanism, releasing the large amounts of virus accumulated. Similar findings are emerging for the baculoviruses (although no Death Protein gene has yet been identified).

RNA viruses like HaSV generally replicate much more rapidly than the large DNA viruses, and it may be this which allows them to grow to high levels before apoptosis renders the host cells of no further value as a replication chamber. In the case of HaSV, we are exploring the consequences of virus-induced apoptosis for the integrity of the midgut, and whether modified or atenuated forms of the virus are still able to elicit this response.

(2) Cell/tissue specificity of virus gene expression & replication

Among RNA viruses, HaSV appears to be unusual in the specificity it displays with repsect to the type of cell it can grow in; the virus appears restricted to larval midgut cells of its host. This specificity is one of its major strengths as a safe biopesticide. While it is very difficult to elucidate the basis of this specificity, since we cannot make the virus grow in cells to study it, work with other small RNA viruses is shedding light ou host (cellular) genes which are involved in replication and which are also likely to be involved in HaSV replication. P. Ahlquist (University of Wisconsiu, Madison) described work on a plant RNA virus, broine mosaic virus (BMV), aud an insect RNA virus, Flock House nodavirus (FHV) with comparable genome

organizations and replication strategies to HaSV. Their approach was to place a yeast selectable marker gene (the *ura*3 gene) on a virus-dependent recombinant RNA molecule (replicon) and select mutagenized ura⁻ yeast for loss of ability to replicate this *ura*3 gene. This work has led to the identification of several yeast genes required for replication of the plant virus; interestingly, two of these do not affect FHV RNA replication. It is likely that this work will enable identification of homologous genes of insects like the bollworm required for replication of HaSV in vivo. This has significant implications for our understanding of virus specificity, virus-induced pathology and countering resistance should it arise.

C. Rice (washington University, St Louis) described the use of a similar approach to obtain mutant forms of an arbovirus (Sindbis virus) able to replicate in mammalian cells (BHK) cells. Previously, it has been possible to grow these arboviruses in cells of their vector, mosquito, but not in vertebrate cells. Again, a dominant selectable marker gene (encoding puromycin N-acetyltransferase for resistance to an antibiotic which inhibits translation) on placed in a recombinant RNA replicon, and virus mutants selected which do not cause cell-death.

A very different approach was described by Ch. Michiels (University of Louvain) to study factors controlling the tissue specificity of a small RNA virus of mice, the Theiler's picornavirus. This work involved exploring the role of a viral gene expression control signal, the internal ribosome entry signal (IRES) in determining which tissues were susceptible to virus infection. The viral IRES controls virus growth within a suceptible cell. The approach adopted by this group was make transgenic mice carrying a bicistronic CAT/IRES/luciferase marker gene construct. With this system, the CAT marker geue is expressed in all cells of the transgenic mouse, but the luciferase reporter gene is only expressed in cells and tissues in which the viral IRES can function. Preliminuary results reported suggest that the tissue-specificity of IRES function (as determiued by luciferase activity) appears age-dependent, being less less selective in young mice, but showing some selectivity in older mice. This approach has considerable potential for insect pest control, since it indicates that IRES signals of insect picornaviruses could be used to direct toxin expression to specific sites within the insect, or to prevent toxin production where it is not desired, e.g. outside the pest during production of viral vectors carrying toxin genes.

(3) Translational control of RNA viruses

RNA viruses with simple genomes like the picornaviruses, which include a number of poorly characterized insect viruses (including at least one we have obtained from H. armigera) and HaSV, have a wide variety of complex effects on the cellular protein translation machinery, these effects may underlie the pathology induced by such "simple" viruses.

Differiug picornaviral mechamisms for translation shut-off were described by N. Sonenberg (McGill University), who first discovered the IRES over 10 years ago. Studying poliovirus, the best known picornavirus, he described cleavage of the cellular trauslation factor eIF4G, a vital component of the cap-binding protein complex required for translation of capped cellular message RNAs; in contrast, the encephalomyocarditis virus EMCV promotes more rapid dephosphorylation of an inhibitor 4E-BP1 which competes with eIF4G to prevent fromation of the cap-binding complex. These mechanisms both prevent host cells from producing their own

proteins; the viral IRES enables the virus to ensure its own genes are translated, since it is independent of the process involving the cap-binding complex.

Influenza virus recruits a cellular stress pathway (p58/hsp40) to regulate translation (M. Katze, University of Washington, Seattle). This allows the virus to avoid the protein translation shut-off (due to a cellular, interferon-induced protein kinase) which it elicits in infected cells.

Plant viruses of the same type as the cauliflower mosaic virus have evolved a complex and still not fully understood means of regulating translation of their genomes. These are unusual in that several genes are translated from a single messenger RNA, a phenomenon otherwise virtually unknown in plants and animals. T. Hohn (Friederich Miescher Institute, Basel) described recent work on the translation of an unusual rice tungro bacilliform virus(RTBV) AUU initiation codon. Translation of this gene is dependent upon a precise ribosome shunt allowing them to skip a large portion of the viral RNA sequence. Ribosomes which translate normally by scanning along the complete viral sequence appear not to recognize this codon. In addition to shedding new light on the basic molecular biology of a significant group of plant pathogens, this work opens new strategies for the control of transgenes expressed in genetically modified plants, including the expression of multiple genes from a single RNA trauscript.

(4) Plant viruses

Replication of tomato yellow leaf curl geminivirus in its whitefly vector *Bemisia tabaci* was reported by H. Czosnek (Hebrew University of Jerusalem). This is the first solid evidence reported for replication by such a virus in insect vectors, a finding which may have significant implications for the control of these important insect pests.

Mapping of aphid acquisition/transmission determinants on a read-through domain of the coat proteiu of beet western yellows luteovirus was described by H. Guilley (IBMP, Strasbourg).

A plant virus, cowpea mosaic virus (CPMV), was engineered so that a 14-amino acid human rhinovirus epitope (HRV-14) was expressed on the surface of the CPMV particles. X-ray crytallographic studies showed that the epitope formed a non-native conformation, allowing generation of antibodies capable of binding, but not neutralizing, HRV particles (V.Spall, J.Johnson and G. Lomonossoff, Scripps Research Institute and John Innes Centre).

(5) Viruses and the immune system

Viruses of vertebrates have evolved a variety of strategies to evade recognition by the host immune system. These were described in a number of excellent presentations. A. Hill (Oregon Health Sciences University) described several mechanisms used by herpesviruses to prevent antigen presentation by class I MHC molecules to cytotoxic T-cells. These include blockage by the virus-encoded HSP ICP47 protein of TAP, the cellular protein which functions as the transporter associated with antigen presentation and is required to transport peptides across the endoplasmic reticulum (ER) for presentation on the cell exterior. In another strategy, the Epstein-Barr virus (EBV) EBNA-1 protein contains a long Gly-Ala repeat preventing it from being processed for antigen presentation; this protein maintains the EBV genome during latency. Murine cytomegalovirus causes class I molecules loaded with peptides to be

retained in the ER, whereas the human cytomegalovirus causes new class I proteins to be degraded through being extruded from the ER into the cytosol.

Although insect viruses do not need to confront a similar host defence apparatus, this work illustrates how complex the host-virus interaction can be and the many levels at which the potential exists for manipulation of such interactions to enhance pathogenicity or modify specificity in pest control strategies.

HIV and the immune system are engaged in a ferocious war of attrition which was described by D. Ho (Aaron Diamond centre for AIDS reserach, NY). It may be possible to clear the body of the virus if all short- and long-term reservoirs are identified and sufficient drugs applied for long enough. Some of these reservoirs are free virus, productively infected CD4+ T-lymphocytes and latently infected CD4+ T-lymphocytes or macrophages.

MAJOR THEMES DISCUSSED AT THE ICE

Insect immunity

The insect immune response was a major topic of discussion at this meeting. While the description of immune responses in a wide variety of insects, inclnding lepidopteran larvae, continnes to be a major focus, studies on *Drosophila* are yielding a wide variety of genes involved in the immune response and cell signalling. Although little is known about the insect immune response to virus infection, or even whether any exists, there were some useful reports at the meeting on aspects of insect biology relevant to understanding how insects respond to pathogens like viruses.

In lepidoptera, a key area of viral pathogenesis is how the larval midgut is disturbed by virus infection. I presented onr work in this area to an audience of insect immunologists who were impressed by the interference of a simple RNA virus with a very limited complement of genes in the normally well regulated process of midgut growth and regeneration. Our work in this area has significant implications for future approaches to pest control.

(a) cell signalling and genes regulating hematopoiesis

Cell-cell cooperation and adhesion in encapsulation of foreign objects (*Microplitis demolitor* eggs by soybean looper {*Pseudoplusia includens*} larvae) (M. Strand, University of Wisconsin, Madison). Encapsulation requires attachment of a layer of granular cells, followed by several layers of plasmatocytes; termination occurs upon attachment of a final layer of a type of granulocytes. Targets for plasmatocytes can be preincubated in medium conditioned by granular cells. Encapsulation is blocked by the cell adhesion recognition sequence RGDS. Plasmatocyte-induced apoptosis is implicated in granulocyte apoptosis terminating encapsulation.

The study of insect genes controlling signalling within the cell has become a very dynamic area of insect research. Considerable effort is devoted to the study of signal transduction pathways regulating production of blood cells (hematopoiesis) in *Drosophila*. C. Dearolf (Dana-Farber Cancer Institute, Harvard Medical School) reported work on JAK (**Janus** protein-tyrosine kinases) which play a central role in signal transduction by phosphorylating STATs (**S**ignal **T**ransducers and **A**ctivators of **T**ranscription). The Drosphila hopscotch gene (*hop*) encodes a JAK. Cell growth factors like the cytokines activate JAKs and STATs. The *Drosophila* JAK pathway is

implicated in the immune response, promoting cell differentiation, proliferation of larval hemolymph phagocytic cells, cell adhesion and melanization as responses to e.g. infection by pathogens.

(b). Immune system genes and mutations causing tumours in Drosophila.

D. Kimbrell (University of Houston) described the elegant use of enhancer detector strains of *Drosophila* in screening for two types of mutations:

(1) genes expressed in the immune system - hemocytes, lymph glands (site of hematopoiesis) and fat body

(2) genes whose expression increases in response to bacterial infection.

This work has already yielded a number of Type (1) genes:
Dorothy (related to *egt)*
wizard (melanotic tumour phenotype)
tolo
viking
Thor (also induced upon infection i.e. Type I and II)
dappled (melanotic tumour phenotype)
Collagen IV (previously known)

(c). Studies on Polydnaviruses

Parasitoid wasps need to avoid recognition and destruction of their eggs by the host immune system when laid in lepidopteran larvae. This is achieved by coating the eggs with virus-like particles of polydnaviruses, which accumulate in the calyx gland of the wasp. These polydnavirus coatings allow the wasp eggs to evade recognition by the immune system of susceptible hosts, so that they escape being encaspulated and destroyed. Moreover, although the polydnaviruses do not appear to replicate in the parasitized host, their genes are expressed and regulate the host response by interference in the juvenile hormone (JH) system.

R. Lanzrein and A. Gruber (University of Berne) provided evidence that the polydnavirus DNA is in fact integrated in the genome of the braconid wasp *Chelonus inanitus.* The integrated DNA is present in both sexes at all stages, and circular excised forms are seen in females (calyx gland) at later stages. These findings imply that polydnaviruses are actually wasp genes amplified in the caly gland and do not replicate by direct copying of their own genetic information, so that they are not viruses in the true sense. Instead, they appear to be gene vectors by which the wasps manipulate gene expression and physiology of the parasitised target. Polydnavirus genes and the wasp venom work together to regulate host larval development by arresting growth and allowing the developing wasp larvae time to complete their own developmental cycle. In view of the increasing interest in the use of parasitoid wasps and components of their venom for biological control of pests, these findings are of great significance.

Other areas of insect biology

Sevarl valuable reports concerned the growth and structure of the lepidopteran midgut. K. Baldwin (Howard University, Washington) described the role played by cell proliferation and differentiation during the 200-fold increase in the surface area of the tobacco hornworm (*Manduca sexta*) midgut. These processes appear to be intimately controlled by intercellular communication via gap junctions, especially

those between the goblet cells and stem cells. Since HaSV disrupts midgut formation while eliciting significant cell proliferation, an understanding of midgut cell biology is vital to explaining its pathology and using this information in exploring alternative, HaSV-derived biological control agenst. U. Klein (Munich) reported on studies of the ATPases which appear to be abundant on the external surface of the goblet cell cavity. This work was of great interest to us because of our finding that HaSV binds to an abundant receptor located at this site. This finding has significant implications for evaluating the possibility of larvae developing resistance to viruses like HaSV. While no talks other than mine discussed the midgut response to virus infection, W. Terra (Sao Paulo) and M. Lehane (Bangor) described its response to infection by bacteria and other pathogens, including the induction of genes encoding anti-bacterial peptides like the defensins.

In another area of insect molecular biology, K. Iatrou (Calgary) described new expression vectors for lepidopteran insects based on cellular actin promoters from the silkworm (*Bombyx mori*). Like other systems based on constitutive promoters from baculoviruses, these new approaches are likely to be of value for the non-host production of viruses like HaSV, just as plant promoters of viral origin are being used in engineering plants to produce HaSV.

SIP MEETING

The meeting of the SIP was characterised by a number of reports on work with engineered baculoviruses, rather than on the ground-breaking work exploring the basic biology of these important biological control agents which featured at the 1994 meeting in Montpellier. The work on baculoviruses presented at this meeting is included in the following summary which covers all three meetings attended.

BACULOVIRUSES AS BIOPESTICIDES

1. Trials of recombinant baculoviruses expressing various toxins.

Recent progress in engineering baculoviruses to express a variety of toxin genes was reported by many groups at all three meetings, including the SIP. This work is summarized in the following table.

Insert toxin gene/promoter	Virus	Results	Laboratory (Meeeting)
JHE.KK/IE-1	AcMNPV	early expression	Guarino (ICV)
AaIT/IE-1	"	early expression, fast kill; larvae smaller than with p10/AaIT	
LqHIT2/IE-1 depressant toxin	AcMNPV	faster kill	DuPont
LqαIT	AcMNPV	faster kill	Chejanovsky
LqHIT2 depressant toxin	"	faster kill	(ICV, SIP)
LqHIT1 excitatory toxin	"	faster kill	
Bt CryIAb	AcMNPV	slight improvement reported	Qi (ICV)
B. mori PTTH	BmNPV	no effect	Maeda (ICE)
egt deletion	"	faster kill	
PTTH+*egt* deletion.	"	12h faster than egt deletion.	
fungal subtilisin-like protease	AcMNPV (pol⁻)	50% less time to kill (pol⁻)	Wood (SIP)

Other work on baculoviruses reported:

ICV:

M. Mikhailov (University of Oxford) reported the construction of new vectors for engineering multigene expression systems into baculoviruses. Up to 11 genes were simultaneously inserted into a single recombinant baculovirus. They were driven by a number of different promoters and inserted at four different sites on the long

baculoviral genome, replacing genes non-essential for baculovirus replication cells (e.g. polyhedrin, p10, p26, p74, egt, DA26).

SIP:

Baculoviruses induce apoptosis (programmed cell death) in infected cells, but carry genes whose products serve to prevent or delay the apoptotic response. This allows the virus time to complete its replication cycle within the cell and increases the virus yield obtained. The protective response may be host specific, as shown by the example of AcMNPV, which normally induces apoptosis in cultured *Choristoneura fumiferana* (spruce budworm) midgut cells. The response is blocked by coinfection with the CfMNPV (G. Caputo, R. Palli, Canadian Forest Service), and may be due to delayed expression of AcMNPV p35 which has an anti-apoptotic function) in these cells, derived from what is not normally a host.

The structure of the ubiquitin/polyhedrin gene cluster in beet armyworm (*Spodoptera exigua*) MNPV baculovirus was reported by J.Vlak et al., (Wageningen Agricultural University, Netherlands), who are sequencing this complete virus genome. Comparison with other baculoviruses whose genomes have been completely sequenced shows evidence for significant rearrangements in order and organization of a number of genes homologous among many baculoviruses. These observations illustrate the plasticity of the baculoviral genomes.

Biological containment of a baculovirus used to control spruce budworm (B. Arif, Canadian Forest Pest Management Institute). These workers are investigating use of genetically modified forms of the CfMNPV baculovirus to control *Choristoneura fumiferana* (spruce budworm). Concern about the dangers of releasing genetically modified viral insecticides carrying toxin genes led them to test strategies for the containment of snch viral control agents upon release, so that they do not persist in the environment. Containment of the CfMNPV is achieved by deletion of the gene encoding the p74 protein, which is essential for horizontal spread of the virus. Since the initial infection of the target insect pest requires the virus to carry the products of these genes, the modified viruses are grown in insect cells either in combination with a complete wild type virus that itself lacks the toxin gene (the co-occlusion strategy) or alone to give, if the polyhedrin gene is absent, virus equivalent to that derived from polyhedra (the pre-occluded virus strategy). The modified virus is initially infectious, but its deficient genome means that progeny virus cannot persist in the environment. Unlike the polyhedrin gene, which has been tested in earlier containment strategies and whose product must be produced in large amounts, only small amounts of p74 are needed during production of genetically crippled virus to render this virus infectious.

A study of whether resistance could be observed in *Spodoptera littoralis* exposed to SlMNPV in lab experiments was reported by Brown et al., NRI, UK. No evidence for development of resistance by this species could be detected.

1997 Annual Report Summary - CSE62C

The major aims of this trip were to attend three international conferences in areas of entomology, insect pathology and virology relevant to the long-term biological control of major cotton pests like *Helicoverpa armigera*. A further major aim was to attend a Management Committee Meeting, hosted by Zeneca Agrochemicals/Seeds (UK), to review progress on the collaborative *Helicoverpa armigera* stunt virus (HaSV) project aimed at exploiting HaSV genes for control of heliothine pests on cotton. This work is partly funded by the CRDC under CSE55C and described in reports concerning that project.

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