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meeting point

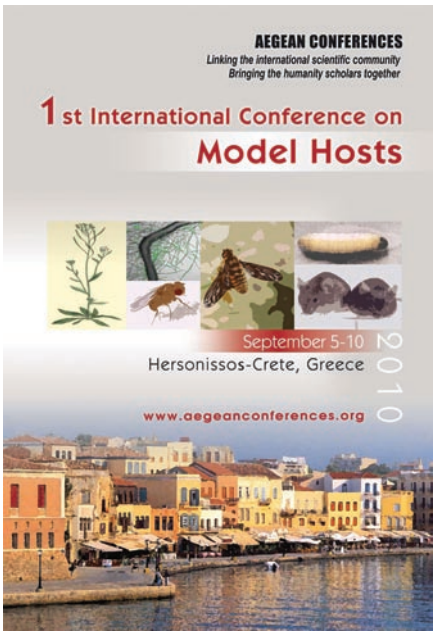
Models to study ancient host–pathogen interactions: lessons from Crete

Terry K. Means & Alejandro Aballay

The Aegean Conferences' first International Conference on Model Hosts took place on the picturesque Greek island of Crete. This meeting was the first of its kind and gathered together international experts who are using a vast array of hosts as models of infection, including worms, insects, mice, fish, rats, humans, squids, pigs, monkeys, protozoa, amoebae and ticks.

On the beautiful Greek island of Crete—the ancient centre of Minoan civilization—the first International Conference on Model Hosts took place in the city of Hersonissos between 5 and 10 September 2010. The organizers, Eleftherios Mylonakis (Massachusetts General Hospital, Boston, USA), Frederick Ausubel (Massachusetts General Hospital), Arturo Casadevall (Yeshiva U., New York, USA) and Michael Gilmore (Schepens Eye Research Institute, Boston, USA), convened an internationally diverse group of scientists to discuss the latest advances in the use of model hosts to understand ancient host–microbe interactions. Between the exciting presentations, the attendees visited the famous Minoan Palace in Knossos that was built around 7,000 BC. The palace itself is an immense structure containing over a thousand rooms, with beautiful frescoes and underground plumbing. The setting could not have been more perfect: the Minoan civilization demonstrated an unparalleled ability to evolve and adapt to its environment, mirroring the evolutionary arms race constantly taking place between microbes and hosts.

Unlike the Minoans, who benefited from the waters around Crete that separated them from potential enemies, multicellular organisms have evolved in direct contact with the micro-organisms that are potentially pathogenic to them. As a consequence, they have evolved a series of weapons and fortifications to protect themselves from attacks. One question in the field is how organisms avoid mounting strong immune responses to non-pathogenic microbes, but actively respond to potentially pathogenic microbes. This is where multicellular model hosts can contribute to our understanding of the battlefield.



At the meeting in Crete, several investigators discussed the latest studies concerning the recognition of pathogens, damage signals and the deleterious consequences of microbial infections, as well as their roles in the activation of immune responses. Evidence was also presented indicating that several of those mechanisms operate not only at the cell-autonomous level, but also at the organismal level.

Arguably, some of the strongest contributions of model systems to the field are related to the discovery that the Toll and immune deficiency (IMD) pathways mediate protection from infections in *Drosophila melanogaster* by activating the production of antimicrobial peptides through NF-κB-like signalling mechanisms (Ganesan *et al*, 2010). This activation of antimicrobial substances requires the recognition of molecules called microbe-associated molecular patterns (MAMPs), which are unique to microbes. However, because MAMPs are also present in non-pathogenic microbes, it has been postulated that recognition of the deleterious consequences of infection is necessary for appropriate immune responses to be mounted against pathogens. Thus, the recognition of damage molecular patterns (DAMPs) as well as MAMPs seems to be necessary for proper activation of the immune system.

It remains unclear whether MAMPs and DAMPs are recognized by the same cells, or whether different cells and tissues recognize them and communicate with each other. Nevertheless, increasing evidence indicates that neuroendocrine signals and the nervous system have a role in the integration of signals from infected sites, to coordinate immune responses. Evidence that the steroidal hormone ecdysone controls the IMD pathway in *D. melanogaster* was presented by Neal Silverman (U. Mass. Medical School, Worcester, USA). The IMD pathway, which is particularly important against Gram-negative bacteria, is activated by the receptor recognition of DAP-type peptidoglycan—a common MAMP in this group of microbes. The engagement of the receptors involved in the recognition of

peptidoglycan elicits the caspase-mediated cleavage of the IMD protein, which allows the interaction of IMD with Lys 63-linked polyubiquitin chains and subsequent upregulation of antimicrobial peptides through the NF-κB-like transcription factor Relish (Paquette *et al*, 2010).

Silverman described studies showing that treatment with the steroidal hormone ecdysone is necessary for robust upregulation of antimicrobials after exposure to peptidoglycan. The data indicate that ecdysone treatment results in the constitutive expression of the cell-surface receptor PGRP-LC, which is involved in the recognition of DAP-type peptidoglycan, resulting in the upregulation of antimicrobial peptides. Ecdysone is produced in the ring gland—so-called because it circles the anterior portion of the digestive system. This gland lies close to the brain and houses the neurosecretory ends of brain cells. The foci of secretion of ecdysone involved in the control of the IMD pathway are unknown, but these results indicate that the nervous system might influence the IMD pathway through ecdysone in *D. melanogaster*.

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Recent studies indicate that specific genes and neurons in the nervous system of the nematode *Caenorhabditis elegans* control both the avoidance of certain pathogens and immune pathways (Styer *et al*, 2008). Alejandro Aballay (Duke U., Durham, USA) presented further evidence indicating that two pairs of neurons actively suppress innate immune responses by downregulating the expression of genes that are part of a non-canonical unfolded protein response pathway (UPR). Interestingly, a phenomenon that Daniel Kalman (Emory U. School of Medicine, Atlanta, USA) has called 'conditioning' is also controlled by the nervous system (Anyanful *et al*, 2009). Kalman demonstrated that brief exposure to enteropathogenic *E. coli* (EPEC) makes *C. elegans*—through dopaminergic neurons—more able to survive a subsequent longer exposure, that would otherwise be lethal. Kalman also presented data indicating that indole produced by EPEC induces conditioning and that protection requires the PMK1 p38 mitogen-activated protein kinase pathway and the DAF2/DAF16 insulin signalling pathway, which control *C. elegans* innate immunity.

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Recognition of MAMPs, virulence factors or metabolic products secreted by pathogens is not the only mechanism involved in the activation of immune responses. Recent evidence indicates that the recognition of DAMPs might be crucial to the activation of the immune system and the identification of pathogens. So far, the specific DAMPs and the receptors that they interact with to activate immune responses are unknown. However, members of the scavenger receptor family recognize DAMPs and are required for innate immunity. It has been demonstrated recently that two *C. elegans* receptors—CED-1 and C03F11.3—and their mammalian orthologues—the scavenger receptors SCARF1 and CD36—mediate defence against microbial infections in the host (Haskins *et al*, 2008; Means *et al*, 2009). Terry Means (Massachusetts General Hospital) presented evidence that CED-1 and C03F11.3 are required for the innate sensing of pathogenic fungi, but not for non-pathogenic ones. Worms deficient in these receptors succumbed to fungal infection more quickly than wild-type worms, which was linked to the decreased expression of several antimicrobial peptides. In mammalian macrophages, SCARF1 and CD36 might function as co-receptors by binding and phagocytosing fungal pathogens to facilitate TLR2 signalling. In a mouse model of cryptococcosis, SCARF1 and CD36 expression were required for the control of fungal burden and cytokine expression. The MAMPs and DAMPs recognized by SCARF1 and CD36 are still unknown, but evidence suggests they are glucan- and mannan-containing molecules, probably located in the fungal cell wall. These data suggest that scavenger receptors might function as TLR co-receptors in pathogen recognition, and they might be targets for therapeutic intervention.

Damage signalling might aid the recognition of pathogens and activate responses against them, but also it seems to activate a process that deals with the damage caused by either the pathogen or the inflammatory response of the host. Dominique Ferrandon (U. Strasbourg, France) emphasized that this process, called endurance, implies that the organism is unable to clear the infection and has to adapt to the damage induced by the pathogen. He presented a whole-genome *in vivo* RNA interference screen that was used to study the mechanisms involved in both endurance and resistance against intestinal infections in *D. melanogaster* (Cronin *et al*, 2009). The data showed that intestinal infection by *Serratia marcescens* triggers the activation of the JAK–STAT signalling pathway, which regulates stem-cell proliferation. The activation of stem-cell proliferation in the intestine might be necessary to compensate for the extensive cell death that takes place in the midgut during infection, thus maintaining epithelial-cell homeostasis.

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Several of the studies in model systems that were presented demonstrated that microbes are important for epithelial homeostasis and organogenesis. Margaret McFall-Ngai (U. of Wisconsin, Madison, USA) highlighted the importance of the effects of microbe–host interactions in epithelial homeostasis. McFall-Ngai's model uses the squid *Euprymna scolopes* and its mutualistic bacterium *Vibrio fischeri* to study beneficial microbe–host interactions (McFall-Ngai, 2008) and the impact of benign microbe–host–symbiont associations on the development of host tissue. Soon after hatching, the host squid is colonized by a cluster of 5–10 *V. fisheri* cells that begin cross-talk communication with host tissues to establish a highly dynamic association. The interaction with *V. fisheri* allows the nocturnal squid to use bacterial luminescence as a counter-illumination mechanism to avoid detection by predators and prey, and to set the stage for subsequent colonization by other bacterial species. McFall-Ngai presented studies indicating that cell-envelope constituents of *V. fischeri* have a key role in the early developmental

events required for colonization. More specifically, derivatives of lipopolysaccharide and peptidoglycan seem to participate in host-organ development. Studies with purified peptidoglycan derivatives and lipopolysaccharide suggest that additional signals from the symbiotic bacterium might also affect morphogenesis.

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The studies presented at this meeting highlighted the many commonalities between microbes and the immune responses of several vertebrate and invertebrate hosts. For example, many of the receptors, cell types and signalling pathways that control immune responses are evolutionarily conserved among hosts. The continuing study of these immune pathways in model hosts will probably answer questions about basic host defence systems and indicate potential therapeutic targets for treatment of infections.

Recently, small invertebrates such as the nematode *C. elegans* have been used as whole-animal model systems for screening new drugs with antimicrobial properties. At this meeting, Frederick Ausubel (Massachusetts General Hospital) and Eleftherios Mylonakis demonstrated how *C. elegans* could be used for the high-throughput *in vivo* screening of chemical libraries for antimicrobial drug discovery. As mentioned above, *C. elegans* can host many microbial pathogens that also infect humans. There are many advantages to performing *in vivo*, live, whole-animal screens to identify antimicrobial compounds. The main advantage over *in vitro* models is that the compounds are tested on live hosts during infection—a strategy that can identify compounds that target microbe-specific virulence factors. Second, the whole-animal approach directly tests the efficacy and toxicity of compounds *in vivo*. Finally, invertebrate screens are less expensive and avoid the ethical issues related to the use of mammalian systems.

Recently, the Ausubel and Mylonakis labs have developed an automated high-throughput *C. elegans* screening assay through miniaturization to 96- and 384-well plate formats. Infected worms are dispensed into individual wells by a large particle sorter, and images of each well are taken to determine worm survival by an automated microscope. By using this approach, Ausubel reported that more than 37,000 small molecules and natural extracts were tested for their ability to increase nematode survival following infection with the Gram-positive bacteria *Enterococcus faecalis*. This screen identified 108 compounds that increased worm survival. Of these, 80 were known antibiotics or had antimicrobial activity, which further validated the efficacy of the screening strategy (Moy *et al*, 2009). Interestingly, 28 compounds were identified with curative activity that were not known antibiotics and had no previously documented antimicrobial activity. At least nine of these compounds inhibited pathogen growth *in vivo* in living worms, but did not inhibit growth *in vitro*, suggesting that they might exert their function by directly regulating the *C. elegans* immune response or inhibiting virulence factors. Indeed, five compounds were shown to activate directly the immune response pathways of *C. elegans*.

Similarly, Mylonakis presented the use of a high-throughput *C. elegans* assay to identify antifungal compounds against *Candida albicans* (Breger *et al*, 2007). This opportunistic pathogenic fungus infects immunosuppressed patients, and strains of this fungus are becoming more resistant to conventional antifungal agents. So far, many antifungal targets have been toxic to the host and amphotericin B—discovered in 1955—is still, clinically, the drug of choice, highlighting the need to identify novel antifungal compounds.

Studies using *C. albicans* mutants defective in hyphal filamentation production demonstrated that in mammals and worms, candida filamentation is required for virulence. Using data from a *C. elegans*–candida *in vivo* screen, Mylonakis reported that more than 1,000 compounds with known pharmaceutical activities were tested for their ability to increase nematode survival after infection with *C. albicans* (Okoli *et al*, 2009). This screen identified 15 compounds that enhanced the survival of *C. elegans* and prevented *in vivo* filamentation of candida. One of these compounds was caffeic acid phenyl ester (CAPE), which also enhanced survival in a murine model of haematogenous candidiasis. Whereas CAPE significantly inhibited filamentation of candida *in vivo*, it only modestly inhibited candida growth *in vitro*. These data suggest that the antifungal activity of CAPE is independent of its ability to inhibit the growth of fungi and that CAPE might function as a virulence inhibitor to fungal pathogens. Together, these studies suggest that high-throughput *C. elegans* antimicrobial discovery screens can be used to identify compounds that could be used clinically.

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To say that the first International Conference on Model Hosts was a success is an understatement; the meeting was well organized, in a terrific location, and the scientific presentations were exciting and thought-provoking. Also, no one is likely to forget the delicious and stomach-bursting dinners held at different local restaurants each night. The next meeting—to be held in two years on the Greek island of Rhodes—will undoubtedly be a hot ticket and should teach us even more about the advances to come from the use of model hosts.

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