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converted β , γ , and δ , but not α biliverdin isomers into the corresponding bilirubins and also displayed flavin and ferric reductase activities, much like the vertebrate biliverdin β reductases. In addition, we found that insecticyanin, a biliverdin IX γ chromoprotein from the hemolymph of *Manduca sexta*, is a good substrate for these dipteran BVRs. The results of these studies are discussed in the light of the possible functions of these enzymes in insect iron and heme homeostasis as compared to their vertebrate counterparts.

Expression studies on *Drosophila melanogaster* G-protein coupled receptors

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A wide range of seven transmembrane spanning (7-TM) G-protein coupled receptors have been cloned and expressed from the fruitfly, *Drosophila melanogaster*. Many of them can be specifically activated by either biogenic amines or neuropeptides and some show a high degree of structural conservation with their vertebrate counterparts. Multiple receptor subtypes have been demonstrated for particular receptor classes. The power of *D. melanogaster* genetics should allow the identification of the functional roles of such receptors. However, it is clear that functional expression studies on such receptors in heterologous expression systems such as insect or vertebrate clonal cell lines or *Xenopus* oocytes provide much information about their potential pharmacology and potential coupling to second messenger systems. A number of examples of such studies will be discussed. Some of the general phenomena that have been investigated include: The concept of the agonist-specific receptor subtypes that couple to different second messenger pathways. This is illustrated by studies on a cloned octopamine receptor (Kawakawa et al., 1990, *Neuron* 2:343-354; Roark et al., 1999, *J. Neurosci.* 13:1325-1330) and on a novel cloned neuropeptide Y receptor (Feng et al., 1999, *Soc Neurosci. Abstr.*, 25:183). Recent expression studies on a novel cloned *D. melanogaster* β -adrenergic-like receptor (Yu et al., 2000, *Society Neuroscience Abstracts*, 26:916) will also be discussed. The completion of the sequencing of the *D. melanogaster* genome makes the identification of potential 7-TM receptor sequences much easier, but functional genomic studies will also be required to assess the physiological roles of these receptors.

Plodia interpunctella 0 1,3 glucan recognition protein Properties of the N terminal carbohydrate binding domain

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Bacteria and fungi possess specific molecular pattern molecules that

form essential structural scaffolding. Typical molecular patterns associated with Gram negative bacteria, Gram positive bacteria, and fungi include lipopolysaccharide, peptidoglycan, and 0 1,3 glucan, respectively. Invertebrate pattern recognition molecules function to stimulate the host innate immune response after encountering such nonself moieties. The pyralid moth, *Plodia interpunctella*, possesses a 0 1,3 glucan recognition molecule (PiPGRP) that may function to continuously survey the hemolymph for the presence of foreign fungal cells. The PiPGRP possesses two putative domains consisting of a novel N terminal carbohydrate recognition domain and a C terminal glucanase like domain, which can be separated by an *in vitro* proteinase treatment. The PiPGRP C terminal domain sequence shares similarity with other recognition proteins and 0 1,3 glucanases from bacteria and a sea urchin, whereas the N terminal sequence is unique to members of the arthropod GRP family and lacks sequence similarity with the 0 1,3-glucanases. The full length PiPGRP and constructs corresponding to 118 and 181 residues from the N terminus and 290 residues from the C terminus of PiPGRP were expressed as recombinant proteins using an *Escherichia coli* heterologous expression system. Circular dichroism (CD) analysis of the 118 and 181 residue N terminal constructs indicate that the recombinant proteins are folded and possess primarily a helical secondary structure. The full length protein binds to insoluble P 1,3 glucan and causes *in vitro* aggregation of Gram positive and Gram negative bacteria, as well as yeast. The 181 residue N terminal construct causes significant aggregation of yeast cells but no aggregation of bacteria. These data suggest that the PiPGRP functions as a pattern recognition molecule in the innate immune system of *P. interpunctella* and that the N terminal domain possesses a carbohydrate binding site necessary for recognition of non self.

Analysis of dietary proteins derived from prey eggs and an embryonic cell line and their effects on the fecundity of *Orius insidiosus*

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The fecundity of the insidious flower bug, *Orius insidiosus* (Hemiptera: Anthracoridae), was poor when reared on a minimal artificial diet (control diet) composed of brewers yeast, soy protein hydrolysate and chicken yolk. Consequently, we supplemented test diets with homogenates of eggs from the Indian meal moth (*Plodia interpunctella*), proteins or lipids extracted from *Plodia* eggs, or an embryonic cell line (PIE) derived from *Plodia* eggs. Test diets were also supplemented with each of three fatty acids identified to be predominant in prey eggs (palmitic, linoleic and oleic acid), bovine serum albumin (BSA), chicken liver, beef liver, or chicken egg white albumin. Diets were compared against an optimal standard, *Plodia* eggs, and the control diet on the basis of the average total number of eggs a female oviposited during her lifetime. Only proteins derived from *Plodia* eggs and the cell line produced significant improvements in fecundity over the control diet at relatively low concentration of protein, indicating the quality of protein is important in selecting

supplements. Proteins extracted from prey eggs and the cell line were further separated by preparative isoelectric focusing and are being evaluated in the artificial diet.

Genomic approaches to the evolution and function of a large multigene family

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The cytochrome P450 gene family is represented by 90 sequences in the *Drosophila melanogaster* genome. Clues to the evolution of this large multigene family are provided by an analysis of the sequences, of their organization on the chromosomes and by comparisons to other species. Strategies used to unravel the functions of these multiple genes in the fruit fly and in other insect species include the production of functional proteins in heterologous systems and DNA microarray analysis of expression patterns.

Molecular characterization of a family of candidate odorant receptors from the malaria vector mosquito *Anopheles gambiae*

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Olfaction plays an important role in many behaviors, including host preference, feeding, mating and socialization of many insects. We are interested in the molecular biology of olfaction as it impacts upon host selection in the major human malaria vector mosquito, *Anopheles gambiae*, where host preference selection makes a significant contribution to the vectorial capacity of this insect. It is hoped that in addition to providing insight into insect chemosensory pathways, a detailed examination of the olfactory signaling cascade in this critically important disease vector insect may lead to novel strategies to reduce the incidence of malaria. We have recently identified and are in the process of characterizing a large family of candidate odorant receptors (ORs) that are presumed to initiate olfactory signaling in *A. gambiae* (AgORs). In some instances, amino acid alignments between AgORs and putative ORs from *Drosophila melanogaster* display significant homology although it is seldom possible to assign orthology between specific receptors from these two highly divergent flies. The possible evolutionary and biological implications of these relationships will be discussed. Furthermore, data will be presented concerning the developmental, spatial and temporal expression patterns of AgORs along with the effect of the initiation of blood-feeding in adult female mosquitoes. Future studies that address questions as to whether host preference is directly

prescribed by the type of odorant receptors expressed in a mosquito's sense organs will be discussed.

Induction of mosquitocidal immunity in mice immunized with *Anopheles gambiae* midgut cDNA

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Vaccines that can kill mosquitoes may have a profound effect toward limiting the transmission of certain mosquito-borne diseases, especially vaccines against African malaria vectors. In an attempt to generate and characterize anti-mosquito immunity, we immunized groups of mice with two individual *Anopheles gambiae* midgut cDNAs that are induced in the midgut upon bloodfeeding; Ag-Aper1 (a secreted peritrophic matrix protein) and AgMuc1 (a midgut-bound mucin). We also immunized two separate groups of mice with an *An. gambiae* midgut cDNA library from bloodfed mosquitoes; one of these groups received a final boost of midgut protein. Humoral and cellular immune profiles were recorded from the immunized mice and cages of *An. gambiae* mosquitoes were fed on these same mice and monitored for rates of mortality and fecundity. We observed consistent and significant increased mortality from mosquitoes that fed on either the AgMuc1 (Muc) or the cDNA library (Lib) immunized mice as compared with controls, but no differences from those that fed on either Ag-Aper1 immunized mice (PM1) or the midgut protein-boosted mice (Lib+Prot). Western blots revealed that all experimental groups produced antibodies that recognized protein corresponding to the cDNA(s) with which they were immunized. However, ELISA measurements of the midgut-specific antibody titers showed that mice immunized with DNA alone produced very low to undetectable antibody titers. Antigen re-stimulation assays using immunized mouse splenocytes showed that the quantity of Type 1 cytokines (TNF- α , IFN- γ) secreted from Muc, Lib, and Lib+Prot groups was significantly higher than either the control groups or the PM1 group. However, the quantity of Type II cytokines (IL-5, IL-10) secreted from the Lib+Prot and PM1 groups was significantly greater when compared to all other groups. Acellular immune sera from each immunization group was pooled and fed *in vitro* to mosquitoes but all failed to increase mosquito mortality. Our results show that mosquitocidal immunity can be consistently generated from midgut DNA immunization and they suggest that this DNA-induced mosquitocidal immunity is cell-mediated.

Foreign gene expression and endogenous gene repression in mosquitoes using recombinant Sindbis virus vectors

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