Innate Immunity in Insects, Function and Regulation of Hemolin from *Hyalophora cecropia*

Katarina Roxström-Lindquist

Stockholm 2002
Abstract. Insects are useful models for the study of innate immune reactions and development. The distinction between recognition mechanisms preceding the breakdown of apoptotic cells during metamorphosis, and the breakdown of cells in response to infections, is unclear. Hemolin, a Lepidopteran member of the immunoglobulin superfamily, is a candidate molecule in self/nonself recognition. This thesis investigates hemolin function and hemolin gene regulation at a molecular level.

We investigated the binding and cell adhesion properties of hemolin from *H. cecropia* and demonstrated that the proteins could homodimerize in presence of calcium. Moreover, a higher molecular weight membrane form of hemolin was present on hemocytes. These results, taken together with an earlier finding that soluble hemolin inhibits hemocyte adhesion, indicated that the secreted hemolin could modulate hemocyte aggregation in a competitive manner in the blood. In addition, hemolin was expressed in different tissues and at different developmental stages.

Since hemolin is expressed both during development and during the immune response, its different regulatory factors must act in concert. We found that the third intron contains an enhancer, through which Dif, C/EBP and HMGI synergistically activate a reporter construct *in vitro*. We concluded that the enhancer is used during infection, since the κB-site is crucial for an immune response. Interestingly, we also found that the active form of the steroid hormone, ecdysone, induces the hemolin gene transcription *in vivo*, and in addition, acts synergistically during bacterial infection. Preliminary *in vivo* results indicate a secondary effect of ecdysone and the importance of hormone receptor elements in the upstream promoter region of hemolin.

To explore the use of *Drosophila* as a genetic tool for understanding hemolin function and regulation, we sought to isolate the functional homologue in this species. A fly cDNA library in yeast was screened using *H. cecropia* hemolin as bait. The screen was not successful. However, it did lead to the discovery of a *Drosophila* protein with true binding specificity for hemolin. Subsequent characterization revealed a new, highly conserved gene, which we named yippee. Yippee is distantly related to zinc finger proteins and represents a novel family of proteins present in numerous eukaryotes, including fungi, plants and humans. Notably, when the *Drosophila* genome sequence was revealed, no hemolin orthologue could be detected.

Finally, an extensive *Drosophila* genome chip analysis was initiated. The goal was to investigate the *Drosophila* immune response, and, in contrast to earlier studies of artificially injected flies, to examine a set of natural microbes, orally and externally applied. In parallel experiments viruses, bacteria, fungi and parasites were compared to unchallenged controls. We obtained a unique set of genes that were up-regulated in the response to the parasite *Octosporea muscadomesticae* and to the fungus *Beauveria bassiana*. We expect both down-regulated and up-regulated genes to serve as a source for the discovery of new effector molecules, in particular those that are active against parasites and fungi.

© 2002 Katarina Roxström-Lindquist
ISBN 91-7265-504-6 pp 1-62
Akademityrck, Edsbruk 2002
Duga eda drepast

An old Icelandic saying (live or let die)
This thesis is based on the following articles, which will be referred to in the text by Roman numerals. Some additional results are presented.


\(^\dagger\) These authors contributed equally to the work.

Reprints were made with permission from the publishers.
## CONTENTS

**ABBREVIATIONS** 8

**INTRODUCTION** 9
- The insect as a model organisms 9

**THE INSECT IMMUNE SYSTEM** 11
- Defense mechanisms 12
  - Hemocyte reactions 12
  - Phenoloxidase and melanization 14
  - Antimicrobial peptides 14
  - Hemolin 16
- Recognition of pathogens 17
  - Danger signals 19

**Signal transduction** 20
- Transcriptional regulation 22
  - Regulation of insect immune genes 24
  - Transcriptional cross-talk with hormones 26

**IMMUNITY DURING DEVELOPMENT** 27
- Infection during development 27
- Control of development 28

**THE PRESENT INVESTIGATION** 30
- Aims of this study 30
- Methodological considerations 30
  - Insect models 30
  - EMSA 30
  - Transfection of eukaryotic cells 31
  - Northern blot 31
  - Yeast two-hybrid system 31
  - GeneChip analysis 32
Results and discussion

Cell adhesion properties of Hemolin

Potential function of Hemolin

Hemolin intron enhancer

Hormone regulation of Hemolin

Hemolin meets Yippee

Yippee as bait

Specificity in immune response

CONCLUDING REMARKS

Future perspectives

ACKNOWLEDGEMENTS

FOR FRIENDS NOT INTO SCIENCE (in Swedish)

REFERENCES

PAPERS I-V
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/EBP</td>
<td>CCAAT/enhancer binding protein</td>
</tr>
<tr>
<td>EcR</td>
<td>ecdysone receptor</td>
</tr>
<tr>
<td>GR</td>
<td>glucocorticoid receptor</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HMG</td>
<td>high mobility group</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>intracellular adhesion molecule-1</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IκB</td>
<td>inhibitor of NF-κB</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>LTA</td>
<td>lipoteichoic acid</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor κB</td>
</tr>
<tr>
<td>PGN</td>
<td>peptidoglycan</td>
</tr>
<tr>
<td>PMA</td>
<td>phorbol 12-myristate 13-acetate</td>
</tr>
</tbody>
</table>
INTRODUCTION

The innate immune system is an ancient form of host defense found in most multicellular organisms, including vertebrates, invertebrates, and plants. It relies on genetically encoded factors that recognize conserved structural patterns on the microbe as nonself (reviewed in Medzhitov and Janeway, 2002). These interactions activate immediate defense responses such as phagocytosis and the production of antimicrobial peptides. In mammals, and probably also in other multicellular organisms, an additional strategy is used for recognition of microbes: the recognition of missing self. Molecular markers expressed on the surfaces of normal uninfected host cells are recognized and the activation of the immune defense is prevented. Lack of these markers on microbes allows the immune system to act specifically against microbial pathogens.

In multicellular organisms, self/nonself discrimination is even more complex. Altered self cells, such as apoptotic and cancer cells, are recognized and eliminated. However, not all nonself microbes are removed. For example, the mammalian gut epithelium is in constant contact with bacteria and bacterial products. Although these factors are normally pro-inflammatory for other cell types, they do not induce an immune response by the gut epithelial cells. However, the epithelial cells do respond to pathogenic bacteria.

In vertebrates, the immune response to microbial pathogens relies on both innate and adaptive immune systems. The innate immune system protects the host in the first minutes/hours and restricts the infection. Guided by the innate immune response, the adaptive immune system is turned on. It is based on specific antigen receptors expressed on lymphocytes that are generated by somatic gene rearrangements and clonal selection. Binding of antigen to its receptor causes a series of cellular events leading to lymphocyte division and the maturation of cells whose functions include antibody secretion, macrophage activation and the killing of infected cells. It takes days to weeks to achieve an adaptive immune response, and during this time the organism is dependent on the quick and broad innate immune system.

The insect as a model organism

The use of insects has contributed significantly to the increasing knowledge of innate immunity for both invertebrates and vertebrates (reviewed in Boman, 1998). The first description of an inducible antibacterial defense system in the fruit fly *Drosophila melanogaster* was described more than three decades ago (Boman et al., 1972). The interest in insect innate immunity increased when H. G. Boman and co-workers identified the cecropins, a group of inducible endogenous peptides with antimicrobial activity. The peptides were first discovered in the giant silk moth *Hyalophora cecropia* (Steiner et al., 1981).
Subsequently, the antibacterial peptides were also found to be important effector molecules in the mammalian immune system.

It is interesting to compare vertebrate and invertebrate innate immunity. This comparison reveals conserved pathways with both similar and dissimilar functions. They both rely to a large extent on certain cell-surface receptors, as exemplified by the Toll family in *Drosophila* and the related Toll-like receptor (TLR) family in mammals (Underhill and Ozinsky, 2002). However, there are also some crucial differences. For instance, evidence suggests that the mammalian TLR proteins detect microbes directly, whereas in *Drosophila* certain pattern recognition molecules can detect microbes and subsequently lead to the activation of the Toll protein. It was recently demonstrated that Toll is important for *Caenorhabditis elegans* feeding behavior (Pujol *et al.*, 2001). This nematode normally feeds on bacteria, but it has the ability to avoid pathogenic bacteria, such as *Serratia marcescens*. When the Toll gene was mutated this behavior was lost. Thus, the Toll protein is part of a defense mechanism in such diverse organisms as mammals, *Drosophila* and *C. elegans*, but functions in significantly different ways in the three organisms.
Insects have developed an efficient immune system, which certainly is partially responsible for the success of these animals. Today, insects have over 1 million species and make up 67% of all animal species (Campell and Reece, 2002). In general, insects have a short life span, produce many offspring and have developed a robust and fast defense. In contrast, mammals are long-lived and encounter the same pathogen many times and develop an adaptive immune response. From this perspective, insects have little use for a long-term adaptive defense. Interestingly, there is evidence of adaptive immunity in long-lived insects such as cockroaches (Karp, 1995). The components responsible for this immunity have, however, not yet been characterized or cloned.

Insects are continuously exposed to potentially pathogenic microbes and eukaryotic parasites, but only a few invaders result in infection. Microbes enter the insect body primarily via the food into the gut and via the air into the tracheal system. Other sources of infections include mating, through wounds in the exoskeleton or with parasitoid wasps injecting their eggs. In the gut, the microbes are challenged by an acidic environment, digestive enzymes and antibacterial lysozyme. For entrance into the body cavity (the hemocoel), they have to pass the peritrophic membrane, a structural protection lining the gut epithelium. The last structural barrier is made up by the epithelial cells covering the insect’s inner and outer surfaces, located in the gut, trachea and under the cuticle. If the microbes succeed in passing these barriers, the infection induces a local response in the host including synthesis and secretion of antimicrobial peptides in the epithelium. In addition, the infection also turns on a systemic response in the hemocoel. The blood cells attach to invading organisms (within seconds to minutes) and stop invasion by phagocytosis. Thereafter, nodules or organized multicellular capsules around the larger parasites are formed (minutes to hours). In addition, the fat body, a functional homologue of the vertebrate liver, produces and secretes effector molecules as antimicrobial peptides into the insect blood (hemolymph). These cellular and humoral responses work in synergy and mediate a successful defense against microbes (Fig. 1).

However, despite this powerful defense, insects can still get sick. By injecting *M. sexta* larvae with bacterial peptidoglycan a “malaise syndrome” (depression) can be induced (Dunn *et al.*, 1994). As early as 6 hours after the injection the consumption of food was decreased. Subsequently, weight loss, a delay in metamorphosis and an increase in the fluidity of the feces developed. The investigators speculated that these symptoms might be part of a mechanism to flush and sterilize the midgut lumen at times when the hemocoel is infected. Moreover, some microbes are harmful to insects. Among the microbial insect pathogens are viruses, bacteria, fungi and protozoa. The Gram-positive bacterium *Bacillus thuringiensis* is maybe the best characterized insect
pathogen, and it has been used in controlling insect pests in agriculture and forestry (Siegel, 2001).

**Fig. 1. Schematic overview of the immune response in insects.**

**Defense mechanisms**

**Hemocyte responses**

The blood cells of an insect are called hemocytes, and they mediate the cellular immune response. There are different populations of hemocytes in the insect performing diverse immune reactions. Hemocytes are normally disaggregated but under the influence of stress-signals from a wound or from a bacterial infection hemocytes are activated and start to aggregate.

A wound is sealed by clotting reactions, where hemocytes and hemolymph molecules interact and form a clot. Bacteria and parasites might also be trapped in the clot, preventing further spread of the infection. Clotting mechanisms are not well characterized in insects, but some coagulation molecules have been identified (reviewed in Theopold et al., 2002). Such
coagulation proteins include lipophorin, a lipid-carrying protein identified as a coagulogen in many insect species (Theopold et al., 2002), and scolexin, a coagulation molecule with sequence similarity to serine proteases, isolated from *M. sexta* (Kyriakides et al., 1995).

Engulfment by phagocytosis is an important primary defense, protecting the host against microbes until further activation of the humoral immune response. This process also eliminates doomed cells to shape organs, so-called apoptosis. In insects, the granular cells and plasmatocytes are the predominant phagocytic cells that can phagocytose viruses, bacteria, fungi and protozoa. In some cases too many microbes enter the hemocoel to be phagocytosed, and then the granular cells degranulate and entrap the pathogens by this material to form nodules. The granular exocytosis is triggered by cell-cell contact and it also releases antimicrobial and cytolytic peptides.

The phagocytosis of foreign and apoptotic cells, while bypassing normal cells, requires specific receptor molecules both on the object and on the phagocytic cell. *Drosophila* flies with hematopoetic disorders develop plasmatocyte tumors that invade and destroy tissues by phagocytosis (reviewed in Gateff et al., 1996). This suggests that the plasmatocytes have a defect in the recognition mechanism.

Encapsulation is another type of cellular response directed against foreign objects that are too large to be phagocytosed by individual plasmatocytes, like eggs from parasitoid wasps. Several layers of hemocytes that are subsequently melanized encapsulate the foreign object. During the initial stage of encapsulation in the moth *Pseudoplusia includens*, granular cells degranulate on the surface of the pathogen, subsequently attracting plasmatocytes (Pech and Strand, 1996). The plasmatocytes attach to the granular cells and to each other forming a multicellular sheath, a capsule. The capsule formation ends when a monolayer of granular cells attach to the last layer of plasmatocytes. Both cell adhesion and signaling between hemocytes are central activities during the capsule formation. The induction of the encapsulation starts with the recognition of the target. In *P. includens* foreign targets are recognized by pattern recognition receptors on granular cells, whereas others are recognized by pattern recognition molecules in plasma (Lavine and Strand, 2001). In a study of hemocyte aggregation in *H. cecropia*, granular cells were always found in the core of aggregates, surrounded of plasmatocytes (Lanz-Mendoza et al., 1996). In *Drosophila*, the plasmatocytes and their modification, lamellocytes, perform encapsulation since granular cells are not present.

Encapsulation is important in a mosquito *Anopheles gambiae* strain, were it acts refractory to infection by the malaria parasite *Plasmodium* (reviewed in Barillas-Mury et al., 2000). It can encapsulate the parasite with melanin in contrast to a susceptible strain that cannot. The molecular mechanism behind the difference between these two strains is not known.
The interactions that result in the binding of hemocytes to foreign objects and to each other during nodule formation and encapsulation are not completely understood.

**Phenoloxidase and melanization**

Phenoloxidase is an enzyme that catalyzes phenols to quinones, generating the black pigment melanin. This enzyme is widely distributed in both prokaryotes and eukaryotes. Three kinds of phenoloxidase exist in insects, namely: granular, wound and hemolymph phenoloxidase. The granular phenoloxidase appears to be involved in the pigmentation of the body wall (Riddiford and Hiruma, 1988). They all seem to be synthesized as inactive prophenoloxidase and are activated by a serine protease cascade. This prophenoloxidase cascade is activated developmentally, in response to stress and to infections (reviewed in Sugumaran, 2001). Also, the cascade is triggered by microbial cell wall components such as β-1,3-glucan, peptidoglycan (PGN) and lipopolysaccharide (LPS), and the cascade is considered to be part of the insect defense system. The activated phenoloxidase oxidizes phenolic substances to the corresponding quinones and their polymerization product, melanin, which is toxic to microbes. Generally, the function of the prophenoloxidase cascade is associated with the production of melanin at the site of injury or around foreign objects entering the hemocoele (Ashida and Brey, 1995).

**Antimicrobial peptides**

After infection in the hemocoele, a battery of different antimicrobial peptides or polypeptides is synthesized and serves as self-produced antibiotics (reviewed in Hetru et al., 1998). The main synthesis occurs in the fat body cells, in epidermal cells at the site of infections, and, to some extent, in hemocytes. The peptides are released into the hemolymph 30 to 60 minutes after the onset of infection and persist for several days during which they kill the invading microbes. Some peptides (apidaecins) appear to be specific for certain species whereas others (defencins) are more general. Most of the peptides, such as cecropins, have a broad specificity. However, there are examples of peptides with narrow specificity: attacin and dipterican are active against Gram-negative bacteria, while drosomycin is active against fungi. The use of antimicrobial peptides is widespread in the animal kingdom.

The antimicrobial peptides act directly on the bacterial cell wall (cecropins) or by interfering with the macromolecule synthesis (attacins). At physiological pH most of the antibacterial peptides have a positive net charge that facilitate the interaction with the cell wall. The peptides that disturb the bacterial cell wall can be subdivided in two major groups (Boman, 1995): peptides that contain cysteine residues and peptides that do not contain cysteine residues. The cysteine residues in the former group form an intra-sulfide bond that results in a rigid structure, while the structures of the peptides in the latter group have a different conformation.
group depend on the environment. Cecropins have no defined structure in solution but an amphipathic \( \alpha \)-helix structure is formed after contact with bacteria. How the peptides kill the microbes is not fully understood. However, many studies have shown that peptide-lipid interactions, rather than a receptor-mediating process, leading to membrane permeation plays a major role in their activity (Shai, 1999). The proposed mechanism for generating permeation of the membrane by amphipatic \( \alpha \)-helical peptides is either transmembrane pore formation via “barrel-stave” or membrane destruction/solubilization via a “carpet” mechanism, leading to the lysis of the bacteria.

The majority of antimicrobial peptides have been isolated from hemolymph, following injury or microbial infections. However, there are also constitutively expressed antimicrobial effector molecules in the reproductive tract. One example is andropin that is produced in the ejaculatory duct of the *Drosophila* male (Samakovlis et al., 1991). Another example are the ceratotoxins that are produced in the reproductive accessory gland of the Mediterranean fruit fly, *Ceratitis capitata*, female (Marchini et al., 1995). These genital tract peptides may protect egg fertilization and offspring survival. In addition, lysozyme, which depolymerizes the bacterial cell wall, is constitutively expressed in the midgut of *Drosophila*, while it is induced in *Lepidoptera* species.

In *Drosophila*, injected Gram-positive and Gram-negative bacteria induce different patterns of antimicrobial gene expression. A natural infection with the fungus *Beauveria bassiana* induces the production of the antifungal peptide drosomycin (Lemaitre et al., 1997). In contrast, the injection of *Beauveria*, results in the production of both drosomycin and the antibacterial peptide dipterin. This systemic response might reflect the injury caused by injection. Also, the injection bypasses the natural order of microbe detection.

The importance of the antibacterial peptides in host defense is supported by the observation that *Drosophila* mutants in the Toll or Imd-pathways, which do not express antimicrobial peptides, are extremely susceptible to microbial infections (Lemaitre et al., 1995; Lemaitre et al., 1996). In addition, using flies deficient in both Imd and Toll pathways but constitutively expressing different antimicrobial peptides demonstrated that the antimicrobial peptides are specific to a certain extent *in vivo*: defencin is the most potent peptide against Gram-positive bacteria, attacin A is active against Gram-negative bacteria and drosomycin is active against fungi (Tzou et al., 2002). Furthermore, it has been demonstrated that attacin A co-expressed with drosocin or dipterincin has a co-operative effect against Gram-negative bacteria. Interestingly, in *Drosophila* domino mutant larvae, which lack blood cells, the antimicrobial genes were fully inducible in the fat body cells, indicating that hemocytes are not essential in the induction signaling (Braun et al., 1998).
**Hemolin**

Hemolin, previously called P4, was originally isolated from the hemolymph of bacteria-injected *H. cecropia* pupae, along with three groups of proteins with antibacterial activity: cecropins, attacins and lysozyme (reviewed in Boman *et al.*, 1985). The early characterization of hemolin described a 48 kDa protein with no antibacterial activity, present in uninfected pupae at a low concentration. However, upon infection it becomes the major inducible protein (Rasmusson and Boman, 1979). Its concentration increases 18-fold (to 7 mg/ml) after 10 days following an *Enterobacter cloacae* injection into *H. cecropia* diapausing pupae (Andersson and Steiner, 1987).

During an infection, hemolin seems to accumulate in the hemolymph, since labeled hemolin when injected into infected and uninfected pupa does not break down. Interestingly, injecting hemolin (0.3 mg/pupae) activates the immune system: hemolin concentration increases 4.5-fold and the antibacterial activity in the hemolymph increases, similar to the response seen during a bacterial infection (Andersson and Steiner, 1987). However, after 2 days the hemolin-induced antibacterial activity declines whereas the bacterially induced activity increases. The fact that hemolin induces its own synthesis suggests that hemolin takes part in cell signaling, however bacteria are needed to obtain a prolonged response.

Besides the induction by an infection, hemolin is also developmentally regulated (Paper III; Trenczek, 1998; Yu and Kanost, 1999). It is synthesized in embryos, the fat body and the midgut during metamorphosis. This expression has previously been described as a constitutive expression in pupae.

Hemolin has been detected in the fat body basal membrane and fat body associated nodules of *Enterobacter cloacae* infected pupae (Andersson and Steiner, 1987). In addition, hemolin is present on or associated with the surface of hemocytes (Paper I; Andersson and Steiner, 1987; Kanost and Zhao, 1996; Ladendorff and Kanost, 1991). Immunodetection in *H. cecropia* has detected hemolin on the surface of naïve granular cells but not on plasmatocytes (Andersson and Steiner, 1987; Raul Bettencourt doctoral thesis, 1999).

The gene encoding hemolin has been cloned in *H. cecropia* and *M. sexta* (Lindström-Dinnetz *et al.*, 1995; Wang *et al.*, 1995). Additionally, several cDNA sequences have been cloned from other *Lepidoptera* species, *Hyphantria cunea* larvae infected with bacteria (Shin *et al.*, 1998), *Bombyx mori* pupae infected with baculovirus (T. Shimada, personal communication), *Antheraea pernyi* pupae infected with bacteria (W. Li, personal communication) and *Lymantria dispar* diapausing eggs (Lee *et al.*, 2002). Analysis of the hemolin cDNA sequence revealed a new member of the Ig superfamily consisting of four Ig-like domains and suggested that hemolin is related to the L1 family of neural cell adhesion molecules (Ladendorff and Kanost, 1991; Sun *et al.*, 1990).
Hemolin, like other cell adhesion molecules, has several potential glycosylation sites. The asparagine-linked glycosylation site in the third Ig domain is highly conserved in hemolin from different species. Hemolin isolated from hemolymph of bacterial injected \textit{H. cecropia} pupae and recombinant protein produced by baculovirus in TN5 cells contains N-acetylglucosamine (Bettencourt \textit{et al.}, 1999). However, \textit{M. sexta} hemolin isolated from the hemolymph of larvae infected with bacteria, from wandering stage larvae and from adults have the same electrophoretic migration and the same masses, suggesting a lack of post-translational modification such as glycosylation (Yu and Kanost, 1999). Different levels of carbohydrates have been detected on hemolin from larvae infected with bacteria and from adults, indicating that the carbohydrates are non-covalently bound. Carbohydrates are believed to be important in the specificity of cell adhesion and immune responses (Lowe, 2001). In addition, carbohydrates are important for self/nonself discrimination.

Hemolin also has several functional similarities to cell adhesion molecules (see paper I). Hemolin from both \textit{M. sexta} and \textit{H. cecropia} prevents hemocyte aggregation stimulated by phorbol myristate acetate (PMA) or lipopolysaccharide (LPS); however, it does not disrupt aggregates already formed (Ladendorff and Kanost, 1991; Lanz-Mendoza \textit{et al.}, 1996). This result indicates that hemolin interacts with molecules on the surface of hemocytes. Moreover, hemolin stimulated the phagocytic activity of \textit{H. cecropia} hemocytes and \textit{Drosophila} hemocytic mbn-2 cells (Lanz-Mendoza \textit{et al.}, 1996). It was also demonstrated that LPS enhanced this activity.

\textbf{Recognition of pathogens}

The recognition of pathogens is an essential component of immunity and is currently being actively investigated. Insects, as other animals, have evolved pattern-recognition molecules that recognize conserved patterns that are found only on evolutionary distant organisms, like bacteria, while being absent on eukaryotic cells (Janeway, 1989). The recognition achieved by these molecules generally leads to humoral and cellular immune responses such as phagocytosis. The conserved patterns can be components of the microbial cell wall, such as peptidoglycan (PGN), that are unique to bacterial cell walls, LPS from the outer membrane of Gram-negative bacteria, lipoteichoic acids (LTA) of Gram-positive bacteria, and \(\beta-1, 3\)-glucans and \(\beta-1, 3\)-mannans from fungal cell walls. These molecules are in some way involved in microbial metabolism and are essential for survival. Thus, they generally cannot be lost or subjected to mutation, which is the case for the virulence factors of microbes (Medzhitov, 2001). Generally, injection of these conserved pattern molecules gives the same kind of immune response as injecting the whole organism. Several potential pattern-recognition molecules capable of binding to the different pattern
molecules on microbes have been isolated and characterized. These pattern-recognition molecules are soluble and/or bound to cell membranes.

The cell walls of bacteria consist of peptidoglycan; the Gram-positive bacteria cell surface is covered by a thick multilayered peptidoglycan, whereas the Gram-negative bacteria have a thin monolayer of peptidoglycan under the outer membrane. Several peptidoglycan recognition proteins (PGRP) have been identified and are activators of innate immune responses in insects and mammals. A PGRP, isolated from the silkworm *Bombyx mori*, binds to Gram-positive bacteria, inhibiting the bacterial growth. This PGRP is also necessary for activation of the prophenoloxidase cascade (Yoshida *et al.*, 1996). In the moth *Trichoplusia ni*, a PGRP is induced by bacterial infection and has also demonstrated strong binding to Gram-positive bacteria (Kang *et al.*, 1998). In *Drosophila* the PGRP family consists of at least 12 proteins (Werner *et al.*, 2000). Recent studies of *Drosophila* mutants reveal a complex family of PGRP proteins, all of which have the PGRP domain. However, some members seem to be essential for different signal transduction pathways (see page 20; Choe *et al.*, 2002; Michel *et al.*, 2001).

LPS-binding proteins in the hemolymph and on hemocytes recognize and neutralize Gram-negative bacteria (reviewed in Gillespie *et al.*, 1997). The Gram-negative bacteria-binding protein, GNBP, isolated from *B. mori* shows sequence similarity to bacterial β-1,3-glucanases and to the vertebrate LPS receptor CD14 (Lee *et al.*, 1996). In *Drosophila*, the DGNBP-1 recombinant protein binds to LPS and to β-1,3-glucan (Kim *et al.* 2000). Like CD14 it can exist both as a soluble form and attached to the cell membrane via a glycosyl-phosphatidylinositol link (GPI-link). The mammalian CD14 is both involved in LPS-induced cellular activation and in the recognition and engulfment of apoptotic cells (reviewed in Gregory, 2000). It can also bind to PGN (Rietschel *et al.*, 1998).

Lectins are proteins that bind to certain sugar residues on cell membranes and thereby lead to cell aggregation. Different types of lectins exist in the insect hemolymph and some are inducible. It is likely that lectins works as opsonins. When bound to LPS on bacteria, lectins can interact with the cell membrane on hemocytes and thereby activate the hemocytes. The LPS-binding protein (LBP) isolated from *B. mori*, is a constitutively expressed lectin that binds to the Lipid A part of LPS (Koizumi *et al.*, 1997). It also induces nodule formation of hemocytes *in vitro*. The lectin hemagglutinin from the snail *Helix pomatia* was found to induce cecropin A1 in the *Drosophila* blood cell-line mbn-2 (Theopold *et al.*, 1996). This lectin binds to hemomucin, a transmembrane protein containing a mucin-like domain in the extracellular part. Hemomucin is expressed in hemocytes and has been found associated with the peritrophic membrane in the gut. The investigators suggest that hemomucin might act as a hemocyte-receptor for lectin-bound microorganisms.
In humans, the plasma mannan-binding lectin (MBL) recognizes carbohydrate structures arranged in a particular geometry presented by a wide range of pathogenic viruses, bacteria, fungi and parasites (reviewed in Petersen et al., 2001). It is found in association with several serine proteases (MAPs) forming the MBL complex. In human, the MBL complex bound to bacteria initiates the activation of the complement cascade, probably an activity performed by the associated serine proteases. Under normal physiological conditions, MBL does not appear to bind to self-surfaces. This lack of binding may be due to the structure of the glycans: the glycans on the surface of animal cells usually end with sialic acid.

There are some reports that cell adhesion molecules, members of the Ig-superfamily, bind to microbes. Hemolin is induced by and binds to bacteria and bacterial membrane components such as LPS (Kanost and Zhao, 1996; Sun et al., 1990). The binding to LPS can be competed away by the Lipid A part of LPS, indicating that Lipid A takes part in the interaction (Daffre and Faye, 1997). Recently, it was demonstrated that *M. sexta* hemolin could bind to LTA, a surface component of Gram-positive bacteria (Yu and Kanost, 2002). LPS and LTA are similar, both containing polysaccharides and lipid compounds. In addition, Yo and Kanost (2002) demonstrated that hemolin binding to surfaces of bacteria and yeast caused the microorganisms to aggregate.

Although the recognition of pathogens is crucial for the immune system, the same interactions can be used for the advantage of the pathogens. The human intercellular adhesion molecule-1 (ICAM-1) normally provides adhesion between endothelial cells and leukocytes after injury. However, various pathogens use ICAM-1 as a cell-surface receptor. For example, the viruses responsible for the common cold, human rhinoviruses (HRVs), initiate infection by binding to ICAM-1 (Staunton et al., 1989). Moreover, erythrocytes infected by the malaria parasite *P. falciparum* bind to ICAM-1 on endothelial cells (Berendt et al., 1992; Ockenhouse et al., 1992).

**Danger signals**

When some insects are wounded, the immune system is induced without the presence of a microbe, suggesting that other signals induces the immune response that are not based on pattern recognition, such as cytokine-like factors. Interestingly, the “Danger model” proposed in vertebrates describes an immune system responding to substances that cause damage, rather than to those that are simply foreign (Gallucci and Matzinger, 2001). Alarm signals are sent by stressed, damaged or parasitized cells to initiate different kinds of immune response in different tissues in response to pathogens. Examples of danger signals are heat-shock proteins, nucleotides, reactive oxygen intermediates, extracellular-matrix breakdown products and cytokines like IFNs.

The redox state of the cell is known to affect the induction of immune genes. Bacterial infections and tissue damage trigger the production of
reactive oxygen species and activates nuclear factor κB (NF-κB) in mammals. In *H. cecropia* the *attacin* gene expression and the Rel transcription factor Cif are activated by H₂O₂ (Sun and Faye, 1995). The *Drosophila turandot A (TotA)* gene, initially identified in the response to a bacterial infection was also found to be induced by other types of stress such as high temperature, dehydration, UV irradiation and oxidative agents (Ekengren et al., 2001).

It is likely that danger signals, together with pattern recognition signals, activate the immune response. The two systems could also be alternatively used depending on the type of defense needed. The outside and the inside of the intact body have different criteria; the gut, lungs/trachea and the skin/exoskeleton are exposed to microbes all the time, whereas the inside of the intact body is kept sterile. If pathogens manage to get inside host cells they are protected to a large extent from the host immune defense. In invertebrates there exist intracellular symbionts, for example the bacterium *Wolbachia*, which live in the cytoplasm of insect cells and apparently do no harm. Intracellular bacteria or parasites cause human diseases, such as tuberculosis and leishmaniasis, in which the bacteria and parasites are hidden inside macrophages. Moreover, the malaria parasites invade the red blood cells.

**Signal transduction**

When the microbes have been recognized, a signal cascade of several reactions leads to the activation of hemocytes and the production of effector molecules. Cell surface receptors are activated and transmit signals into the cell that result in the transcription of genes encoding for effector molecules, so call immune proteins.

The activation of immune reactions seems to be dependent on protease activity: protein degradation is generally important during other physiological processes such as in metamorphosis. Serine proteases play crucial roles in hemolymph coagulation, in the PPO cascade and in the activation of the Toll ligand spaeztle (see page 21). To control the destructive protease activity, serine protease inhibitors (serpins) are produced. In *Drosophila*, the sequence of the genome revealed a surprisingly high number of predicted protease genes, and in genome-wide analysis of immune response both proteases and serpins were up-regulated (Paper V; De Gregorio et al., 2001; Irving et al., 2001).

Insects can distinguish and respond differently to diverse microbes and ways of infection. Phenotype studies of different *Drosophila* mutants revealed two different signaling transduction pathways responsible for the induction of the antimicrobial genes in systemic response (Fig. 2, Lemaitre et al., 1996). The Toll pathway responds primarily to infection by Gram-positive bacteria and fungi, whereas the Imd (“immune deficiency”) pathway responds primarily to Gram-negative bacterial infections. Interestingly, septic injury activates both signaling pathways. In *B. mori*, the *cecropin B* gene is expressed
in response to bacteria but not fungi, indicating that the moth can distinguish bacteria from fungi (Taniai and Tomita, 2000).

The Toll receptor was initially discovered as essential for establishing the dorsoventral body axis during *Drosophila* embryonic development (Hashimoto *et al.*, 1988). Later, this receptor has been shown to be involved in innate immunity (Rosetto *et al.*, 1995; Lemaitre *et al.*, 1996). The Toll receptor ligand is an extracellular cytokine-like peptide called spaetzle. It was shown that in necrotic mutants (*nec*) affecting the serine protease inhibitor (serpin) the spaetzle is predominantly present in its cleaved form and drosomycin is constitutively expressed (Levashina *et al.*, 1999). The expression was abolished after introducing the wild-type serpin into a *nec* mutant background. In addition, the Toll pathway was not activated in a spaetzle mutant upon an infection.

In a genetic screen for *Drosophila* mutants unable to produce the antibacterial peptide drosomycin, the mutant *semmelweis* (*seml*) was identified (Michel *et al.*, 2001). Normally drosomycin is generated after activation of the Toll pathway in response to infection of Gram-positive bacteria or fungi. The *seml* mutant can no longer activate drosomycin in response to Gram-positive bacteria, but can do so in response to fungi. So, it seems that the two microbes are initially detected in different ways although both microbes work through the Toll signal pathway. In *seml* mutants, the gene encoding a soluble peptidoglycan-recognition protein, PGRP-SA, is inactivated. The current model is that PGRP-SA recognizes peptidoglycan in the cell wall of Gram-positive bacteria; a serine protease is triggered to activate spaetzle, which turns on the Toll pathway. Recent mutant studies by Choe *et al.* (2002) revealed that the membrane-bound PGRP-LC was necessary for induction of antimicrobial peptides in response to both Gram-positive and Gram-negative bacterial infections and for the proper activation of the Rel/NFκB transcription factor Relish. Also, using RNA interference (RNAi) it was shown that PGRP-LC was required for the antimicrobial response to both peptidoglycan and LPS in mbn-2 cells. Choe *et al.* suggest that PGRP-LC may bind directly to peptidoglycan and to LPS, or alternatively, to another pattern recognition receptor that has bound LPS.

The epithelial antimicrobial response and the systemic response are different. It seems that during an epithelial response in *Drosophila*, the antifungal peptide drosomycin is a target gene for the Imd-pathway instead of the Toll-pathway and the response produces a relatively small spectrum of antibacterial peptides (Tzou *et al.*, 2000).

In *Drosophila*, the Toll and Imd pathways use different Rel/NFκB transcription factors, Dif or Dorsal and Relish respectively (see page 24, Fig. 2). The Toll intracellular signaling is initiated by the intracellular domain of Toll, which shows sequence similarity to the corresponding region of the mammalian interleukin 1 receptor (IL-1). This domain interacts with the death domain (DD)
region on different proteins: a Drosophila homologue of MYD88, tube and pelle. Besides the DD, pelle also contains a serine-threonine kinase domain. These interactions lead to the phosphorylation of the inhibitor cactus and activation of Dif/Dorsal.

The Imd-pathway is triggered in response to Gram-negative bacteria through an unknown receptor. The imd gene product is intracellular and contains a death domain with sequence similarity to mammalian tumor necrosis factor-α receptor interacting protein (RIP; Georgel et al., 2001). Although the precise signal transduction has not been solved yet, this pathway involves the protein kinase dTAK1, the IκB kinase protein complex dIKK-β/IKK-γ and the caspase dredd that results in the cleavage of Relish and the induction of the antimicrobial genes dipterin and drosocin.

In H. cecropia, PMA, an activator of protein kinase C (PKC), induces immune genes in the fat body. This demonstrates a possible involvement of phosphorylation in the signaling pathway leading to the activation of the Rel transcription factor Cif. Interestingly, when hemolin was added to hemocytes, the PKC activity increased and the tyrosine phosphorylation pattern was affected (Lanz-Mendoza et al., 1996). This is an additional observation indicating hemolin involvement in immune gene activation/signaling.

The pathways that regulate Rel/NF-κB in mammals and Drosophila are similar (Fig. 2; Khush et al., 2001). In mammals there are at least ten Toll-like receptors (TLRs), which sense a large spectrum of microbial patterns, and which subsequently activate NF-κB. In contrast to Drosophila Toll, mammalian TLRs directly interact with microbial patterns, although they do so in association with co-receptors or associated proteins (Medzhitov, 2001). TLR2 and TLR4 are essential for the immune response to peptidoglycan and to LPS, respectively. To achieve a highly efficient LPS-induced response, LPS is bound by LPS-binding protein in serum, which delivers LPS to CD14, which subsequently associates with a complex that includes TLR4 and MD-2. Additional ligands for TLR2, TLR4 and other TLRs are constantly being discovered. The current model is that the immune cells use multiple TLRs to detect several features of a microbe simultaneously (Underhill and Ozinsky, 2002), activating a common signal cascade that triggers NF-κB.

Transcriptional regulation

Gene regulation plays a key role in almost every biological process. The genes that are expressed in a cell determine its characteristics and phenotype and subsequently form the identity of an organism. The regulation of gene expression varies in different stages allowing fine-tuning in time, space and the amount of needed RNA and proteins.
Fig. 2. Conserved signal transduction pathways in *Drosophila* and mammals. Recognition of microbes results in a signal cascade, leading to the activation of Rel/NF-κB and the induction of different immune genes (Khush et al., 2001; Underhill and Ozinsky, 2002).
The most common and an important level of control is regulating transcription. Defects in transcriptional regulation are responsible for many human diseases.

Activators, repressors and mediators modulate the level and the specificity of transcription of eukaryotic genes. To activate a gene the activators, consisting of both transcriptional factors and architectural proteins, bind to enhancer elements and form enhancer complexes, known as enhanceosomes (Maniatis et al., 1998). In many eukaryotic genes activators bind to sites distant from one another and the DNA bends and/or forms a loop in order to form the enhanceosome. Transcription is initiated when the mediator complex bound to RNA polymerase interacts with the enhanceosome.

Many transcription factors can be activated by several signals from the environment and are capable of activating or repressing many different genes. The vertebrate transcription factor NF-κB is induced by over 150 different stimuli and participates in the regulation of over 150 target genes whose functions are found in the immune response and in the general stress response (Pahl, 1999). How can NF-κB confer its specificity? Upon a given signal, part of the specificity is achieved through forming unique enhancer complexes of activators binding to the distinct set of enhancer elements in the regulatory region of genes. In the case of Rel/NF-κB proteins additional specificity is achieved by the selective activation and binding of individual Rel proteins.

Processes like the immune response and development depend on precise regulation. In an immune response against a bacterial infection the organism switches on genes necessary for its protection. However, this response needs to be tightly regulated, since an uncontrolled response may lead to shock for the organism, such as septic shock in humans.

**Regulation of the insect immune genes**

The promoters for several antibacterial protein and peptide genes in insects are regulated by transcription factors similar to those involved in the mammalian acute phase response stimulated by interferon and interleukins. Sun et al. (1991), discovered that antimicrobial protein and peptide genes contain a motif with homology to the mammalian κB motif, which is the binding element of the Rel transcription factor NF-κB. Later they showed that a κB-binding factor is present in extract prepared from induced H. cecropia pupae, and they named this the Cecropia immunoresponsive factor (Cif). Cif shares several properties to the Rel/NF-κB family of transcription factors (Sun and Faye, 1992).

The Rel/NF-κB transcription factors are bound to inhibitory proteins of the IκB family, alternatively containing their own IκB domain, that sequester the factor in the cytoplasm by masking the nuclear localization signal. Upon activation, the signaling pathway results in the degradation and dissociation of the inhibitor, and the Rel factor can enter the nucleus and activate specific genes.
This results in a quick response since the Rel/NF-κB factor is present in the cytoplasm and is kept in a stand-by position.

Induction of antimicrobial peptide synthesis has been studied in detail in *Drosophila*. The *Drosophila* genome encodes three Rel proteins, Dorsal (initially discovered for its role in the dorsoventral patterning in the embryo), Dorsal related immunity factor (Dif), and Relish. Each has a common Rel-homology domain that is responsible for dimerization of the protein and DNA binding. The Rel factors can form homo- or heterodimers, allowing further modification of the activation of gene expression. It was shown in stable transfected S2 cells that different combinations of Rel proteins preferred different target immune genes. Relish/Dif and Relish/Dorsal heterodimers gave the strongest activation of the *drosomycin* and *defencins* genes, respectively (Han and Ip, 1999).

In adults, Dif is the predominant transactivator in the immune response against Gram-positive bacteria and fungi activated through the Toll pathway. However, in larvae, Dorsal can substitute for Dif in regulating the *drosomycin* gene (Rutschmann et al., 2000). Flies that carry point mutations in the gene coding for Dif are susceptible to fungal but not to bacterial infections (Rutschmann et al., 2000). Dif is crucial for the induction of the antifungal peptide drosomycin. Genome-wide analysis of *Drosophila* immune responses suggests that Dif activates the transcription of several hundred genes (De Gregorio et al., 2001; Irving et al., 2001).

Relish is the transactivator in the response against Gram-negative bacteria through the Imd pathway (Fig. 2). Using P-element mediated mutagenesis, the Relish mutant flies were found to be very sensitive to infection (Hedengren et al., 1999). Relish is important in the induction of both antibacterial and antifungal peptides, but in particular in the induction of diptericin. Interestingly, the mutants did not affect the cellular immune reactions such as phagocytosis, encapsulation and melanization. Unlike the other *Drosophila* Rel factors, Relish carries its own IκB domain, i.e. ankyrin repeats that are endoproteolytically cleaved upon signal, allowing for its nuclear translocation (Stoven et al., 2000). Another difference is that Relish, but not Dif and Dorsal, is induced in bacteria-infected flies and by LPS in S2 cells (Dushay et al., 1996; Han and Ip, 1999).

In the flesh fly, *Sarcophaga peregrina*, the *Sarcophaga*-derived Rel/ankyrin molecule (SRAM) also contains ankyrin repeats, but it is present in the nucleus irrespective of immune stimuli (Shiraishi et al., 2000). SRAM cannot activate the *Sarcophaga* lectin gene in the *Drosophila* mbn-2 cells but enhances the activity when co-transfected with Dif. The investigators speculate that SRAM could be a modulator in the expression of immune genes.

Consequently, the induction of some immune genes is dependent on a certain pathway and Rel factor. However, it is likely that interactions between pathways are achieved by heterodimerization among various Rel proteins.
Moreover, different co-activators modify the immune response activated by Rel/NF-κB. Transcriptional control of the *Drosophila cecropin A1* gene depends on three elements: RI-, κB- and GATA-site (reviewed in Engström, 1998). The κB-site works as a regulatory switch in response to an infection, whereas the GATA-site mediates tissue specificity upon binding of the GATA factor serpent. The GATA-site and serpent are required for *CecA1* induction in the larval, but not in the adult, fat body, and in embryonic yolk but not in embryonic epidermis (Petersen *et al.*, 1999; Tingvall *et al.*, 2001a). The factor binding to the R1 element is still unknown, although, the R1 element does have some sequence similarity to the κB-site (H. Uvell and Y. Engström, personal communication). The promoters of several inducible immune genes, also from other insects, possess GATA-sites in proximity to the κB-like sites (Kadalayil *et al.*, 1997).

**Transcriptional cross-talk with hormones**

Hormones modify the regulation of genes by binding to their nuclear hormone receptors. In mammals, steroid hormones and in particular glucocorticoids (GC) inhibit the inflammatory reaction and suppress the immune system. Cortisone is a glucocorticoid used as treatment for many inflammatory diseases. GCs are the ligands for the glucocorticoid receptor (GR), which controls transcription by two major modes of action. It binds as a dimer to regulatory elements in the target gene promoter, and it also modulates the activity of other transcription factors independently of direct DNA contact, a process called cross-talk (reviewed in Henrich *et al.*, 1999). These protein-protein interactions mainly result in inhibition of transcription, but synergism is achieved as well.

Transcription factors such as NF-κB/Rel, the activator protein-1 (Ap-1) and CCAAT/enhancer binding protein (C/EBP) are involved in cross-talk with steroid receptors (Henrich *et al.*, 1999). Regulation of genes which code for essential mediators in inflammation, such as interleukins and interferons, and adhesion molecules such as ICAM-1 is regulated by Ap-1, C/EBP and, in particular, NF-κB. Not only does GR inhibit NF-κB activity by protein-protein interaction, GR also induces the production of IκBα in some specific cell types and it may also inhibit NF-κB mediated transactivation by competing for limited amounts of coactivators (Almawi and Melemedjian, 2002). Repression of these transcription factors by glucocorticoid receptors should to some extent downregulate the expression of the inflammatory mediators.
IMMUNITY DURING DEVELOPMENT

Infection during development

The complete metamorphosis of holometabolous insects such as *H. cecropia* and *Drosophila* demands remarkable regulation and organization in order to create completely new tissues and to take care of old ones. It is energy-consuming but a strategy for the insect’s survival. It is likely that the insects are more susceptible to infection during metamorphosis and therefore prepared to encounter unwanted microbes.

During development, the cellular immune reactions by the hemocytes are modulated. In most insect species, early larval instars are less competent to encapsulate parasitoid eggs and during larval development in *B. mori* the phagocytosis of goose erythrocytes by granular cells is enhanced (reviewed in Gillespie *et al.*, 1997). Furthermore, the phagocytic activity of *Drosophila* mbn-2 hemocyte cells is enhanced after stimulation with the steroid hormone 20-hydroxyecdysone, 20E (see page 28; Dimarcq *et al.*, 1997).

Several immune proteins are also developmentally regulated. Hemolin in *H. cecropia* and in *M. sexta* is expressed differently during development (Paper III; Trenczek, 1998; Yu and Kanost, 1999). Other examples are cecropins from *Drosophila* (Samakovlis *et al.*, 1990) and sarcotoxin I, a cecropin homologue from the flesh fly *S. peregrina*, which are expressed in early pupae (Natori, 1990). Also, the prophenoloxidase 1 gene is regulated by 20E in the malaria vector *A. gambiae* (Ahmed *et al.*, 1999). Moreover, an antibacterial protein activity was identified in the lumen of the newly differentiated pupal midgut of *M. sexta* (Dunn *et al.*, 1994). The amount of different immune related factors such as lysozyme, immunoreactive hemolin and prophenoloxidase/phenoloxidase activity rose directly after the pupal midgut differentiated. During the pupal stage of *S. peregrina* an antibacterial serine protease homologue is expressed in a temporary organ called the yellow body, later developing to the adult midgut (Tsuji *et al.*, 1998). Besides protease activity it also exerted an antibacterial activity. It is supposed that this protease is involved in the larval disintegration and, at the same time, kills the larval normal flora when the larval midgut is destroyed.

The *Drosophila* gene *CecA1* inducibility has been studied during development of embryo and larvae (Tingvall *et al.*, 2001a). Transgenic fly strains carrying *CecA1* upstream region fused to a LacZ reporter gene was analyzed. Injection of LPS or bacteria showed that the *CecA1* was inducible in the yolk cells of the embryo. Subsequently, *CecA1* was induced in the embryonic epidermis and persists throughout the larval development. The fat body becomes immune-competent during the first instar larvae (Tingvall *et al.*, 2001b).
The two processes, immunity and development, use similar mechanisms and share the same proteins for both similar and different purposes. For example, in *Drosophila* the Toll signaling pathway is used in embryo development and in activating the immune response. Furthermore, during infections and development the insect takes care of unwanted cells by phagocytosis; apoptotic cells during development and invading pathogens during infection. Cell adhesion molecules play an important role in mediating communication during immunity and development.

**Control of development**

The life cycle of holometabolous insects starts with the zygote embryo developing into a larva. The larvae increase in size by feeding and molt several times in a need for a larger cuticle. At the last larval stage metamorphosis occurs, the larvae are transformed into adults via a pupal stage. The pupal stage persists for different periods of time depending on the insect. Most of the larval tissues are broken down by programmed cell death and replaced by adult structures that develop from imaginal progenitor cells.

Insect molting and metamorphosis are controlled by a class of steroid hormones known as ecdysteroids and juvenile hormones (JHs), which are related to vertebrate sex hormones. Ecdysteroids initiate and coordinate the molt and JHs maintain the juvenile stage, preventing metamorphosis (Riddiford *et al.*, 2000). A JH membrane or nuclear receptor has not been isolated.

In most insects, the active ecdysteroid appears to be 20-hydroxyecdysone (20E), hydroxylated ecdysone. Like other steroid hormones, ecdysteroids act by binding to nuclear hormone receptors, which are ligand-dependent transcription factors. In *Drosophila*, as well as in other insects such as *M. sexta* and *B. mori*, the binding of 20E to the heterodimer of ecdysteroid receptor (EcR) and ultraspiracle (USP) activates a small group of early genes. These genes encode transcription factors that attenuate their own activity while driving the expression of a large group of late genes (Fig. 3; reviewed in Cherbas and Cherbas, 1996; Thummel, 1997). Relatively little is known about the regulation of these late factors.

In *M. sexta* two isoforms of USP exist. During the larval and pupal molt, a switch from USP-1 to USP-2 occurs in the epidermis due to the rising titre of ecdysteroids (Jindra *et al.*, 1997). MHR3, a homologue to the vertebrate retinoid orphan receptor (ROR) gene, is activated by a heterodimer consisting of EcR-B1 and USP-1, while USP-2 prevents this action in *M. sexta* GV-1 cells (Lan *et al.*, 1999). Several orphan receptors have been cloned and, by definition, their putative ligand and target genes are not known.
Fig. 3. Ecdysone regulation of gene expression during metamorphosis. Binding of the activated ecdysone nuclear receptor complex: 20E, EcR and USP to the ecdysone receptor element (EcRE) activates transcription of primary-response (early) genes. These newly synthesized transcription factors repress their own transcription while activating secondary-response (late) genes.
THE PRESENT STUDY

Aims of this study

Study of the insect immune system has revealed effector molecules against microbes and signal pathways with striking similarities to mammalian innate immune response. However, the molecular mechanism behind the recognition of the infecting agent is not completely understood. Hemolin is one candidate for such a recognition molecule. The aim of my thesis work has been to use *H. cecropia* as a model to increase our knowledge about hemolin function and regulation. In addition, I have used *Drosophila* as model to examine the general specificity in insect innate immune response.

Methodological considerations

Insect models

In my thesis work, I have used different model systems and a variety of techniques to study immunity and development. The giant silk moth *Hyalophora cecropia* and the fruit fly *Drosophila melanogaster* have been my main insect models. One advantage with the *H. cecropia* pupa is that it has a long diapause with low metabolism, making it ideal for induction studies. In addition, the giant *H. cecropia* moth generates much more material than *Drosophila*. The pupa contains at least 1.5 ml hemolymph and is 5000 times larger than the fly (Faye, 1990). However, *H. cecropia* generates only one generation/year while *Drosophila* generates around 40 generation/year, making the flies a better statistical material. Today, *Drosophila* is a powerful model with a fully sequenced genome coupled with advanced genetic tools. To obtain *H. cecropia* pupae, we are normally dependent on American dealers, whereas we easily rear *Drosophila* in our laboratory. Notably, when studying immunity apart from the type of infecting agent, the developmental stage of the insect and infection procedure (injected, pricked or fed) influences the outcome of the experiment.

Electrophoretic mobility shift assay, EMSA: Papers II and III

To analyze the regulatory elements involved in the immune and developmental regulation of *hemolin* gene expression, two methods were used: EMSA and transfections of cell lines. Most of the transcription factors contain domains capable of binding selectively to a particular DNA sequence, where some bases are crucial for binding. EMSA is a simple, quick and sensitive method for analyzing the DNA binding potential of transcription factors (Garner and Revzin, 1981). Proteins that bind specifically to a radioactively end-labeled DNA fragment retard the mobility of the fragment during electrophoresis on a nondenaturing polyacrylamide gel. The sequence specificity of the protein-DNA
interactions is examined by including unlabeled probes in the binding reactions. Also, by including antibodies in the binding reactions, the identity of the binding protein can be determined. We have used this technique to analyze the putative regulatory elements in the hemolin gene, and their ability to bind transcription factors. We have studied factors obtained from nuclear extract preparation of the fat body of normal, bacterial injected or 20E injected H. cecropia pupae. Most experiments have been performed with crude extract, although some experiments have been performed with Cif purified by affinity chromatography.

**Transfection of eukaryotic cells: Paper II**

In order to study the hemolin gene regulatory element, Drosophila blood cells mb-n-2 (malignant blood neoplasm-2; Gateff *et al.*, 1980) and embryonic cells SL2 (Schneider Line-2; Schneider, 1972) were transfected with different reporter gene constructs. The reporter gene was under the control of DNA regions of interest (Fig. 3, paper II). Transient transfection is a commonly used technique to introduce plasmid DNA into tissue culture cells. The DNA is taken up by the cell and persists in the nucleus for several days before it disappears. The transfected DNA is transiently expressed by the regulatory factors of the cell or by cotransfected transcription factors. *In vitro* cell lines are important tools for studying the function and regulation of genes; however, one must keep in mind that the cells are abnormal in that they allow for permanent growth. In addition, it is sometimes hard to study gene regulation without having identified the nuclear proteins and co-factors involved.

**Northern blot analysis: Papers III and IV**

In order to monitor the size, the induction and the developmental expression of the hemolin and yippee gene, total RNA was prepared and analyzed by Northern blot (Alwine *et al.*, 1979). The RNA is separated on a denaturing gel and transferred to a solid support such as a nylon membrane. The mRNA is detected by hybridizing to a radioactively labeled probe complementary to the mRNA of interest. Northern hybridization analyzes the relative amount and size of transcript. However, the method does not distinguish between changes in the rate of transcription induction and differences in mRNA turnover. For this purpose, you need to do a transcriptional run-on assays in intact nuclei. RNase protection is an alternative method to Northern blot for following the expression of a gene.

**Yeast two-hybrid system: Paper IV**

We used a two-hybrid screen searching for hemolin-interacting protein, mostly with the aim of cloning hemolin in Drosophila, since we know that hemolin can form dimers. The yeast two-hybrid system gives the possibility of analyzing protein-protein interactions (Fields and Song, 1989). Many eukaryotic transcription activators have at least two distinct functional domains, one that
directs binding to a specific DNA sequence and one that activates transcription. In the system I used, the protein of interest (bait) has been fused to the bacterial DNA binding domain LexA and the protein in question (prey) has been fused to the transcription activation domain B42 (Golemis et al., 1996). The interactions between the two proteins are detected by reporter gene activity as a result of the reconstruction of a functional transcription factor. To eliminate false positives, the prey is isolated and retransformed in new yeast cells, and the inserts are swapped so that the prey is fused to the DNA binding domain and vice versa. One advantage of the two-hybrid system over, for example immunoprecipitation, is that in a search for unknown interactions, cDNA of the interactive protein genes are already cloned.

One problem when using the two-hybrid system to study immunoglobulin (Ig)-like molecules is that the environment in the yeast cell might be too reducing for disulfide bridges to form. Normally, Ig molecules achieve their oxidized disulfide bonds in the endoplasmic reticulum (ER) before they are exported out of the cell. Cytoplasmic proteins in higher cells do not generally utilize these disulfide bonds as a stabilizing force. However, hemolin is present also in the cytoplasm of oocysts (Bettencourt et al., 2000).

**GeneChip analysis: Paper V**

In order to compare the immune response of *Drosophila* flies to various naturally infecting microbes, we used GeneChip technology. The flies were infected orally and the gene expression was analyzed on GeneChip after 24 hrs. We used Affymetrix *Drosophila* GeneChips (https://www.affymetrix.com), which contain 25-mer oligonucleotide probes in 14 probe pairs per gene, representing almost 14,000 genes predicted from the annotation of the *Drosophila* genome of August 2000. Total RNA was prepared from two separate pools of 100 infected flies for each infection. The RNA was amplified, biotinylated labeled and hybridized to the GeneChip by the Affymetrix core facility at NOVUM. After scanning the GeneChip the results were normalized. By using the Affymetrix software Mas 5.0, we compared the results from replicates of each infection with the results from replicates of normal flies, and isolated those genes that were increased in all four comparisons.

It is worth noting that the GeneChip does not cover the whole *Drosophila* genome. However, the chip seems to be accurate and sensitive: earlier identified immune genes were also found to be induced using the GeneChip (De Gregorio et al., 2001) and the induction of antibacterial peptides correlated well with earlier studies based on Northern blots (Irving et al., 2001).
Results and discussion

**Cell adhesion properties of Hemolin (Paper I)**

The cell-cell contact is critical for the development and the maintenance of multicellular organisms. Cell adhesion between two cells is either stable, as in organized epithelial tissues, or induced, as in the immune response. It is essential in cellular immune responses such as encapsulation and nodule formation (Johansson, 1999). One of the major families of cell adhesion molecules (CAM) is the immunoglobulin super family (IgSF), which mediates cell adhesion between blood cells and cells both from endothelial and nerve system, resulting in intracellular signaling. Hemolin is structurally related to cell adhesion molecules, in particular to neural cell adhesion molecules. It has been shown that hemolin influences cellular immune mechanisms in *M. sexta* and in *H. cecropia* (Faye and Kanost, 1998).

We wanted to further investigate the cell adhesion properties of hemolin. Hemolin is associated with the surface of hemocytes (Andersson and Steiner, 1987). Using flow cytometry, the presence of hemolin on the surface of naïve hemocytes was confirmed (Fig. 1, Paper I) and encourages the isolation of a membrane-associated form of hemolin. A 52 kDa form of hemolin was immunoprecipitated from a membrane preparation of hemocytes and from *Tricoplusia ni* TN-5 cells infected with baculovirus expressing recombinant hemolin (Fig. 2, Paper I). It was not determined how hemolin is bound to the membrane, but it is clear that the higher molecular weight is caused by a post-translational event. Since glycosyl-phosphatidylinositol (GPI) specific enzyme treatment did not diminish the size of the membrane form (data not shown), the modification is likely to be of another type of hydrophobic group, such as a palmitoyl or a farnesyl group. Furthermore, recombinant hemolin could bind to surface of hemocytes and TN-5 cells not expressing hemolin, forming protein complexes that were not dissolved under reducing conditions (Fig. 3, Paper I). Also, recombinant hemolin was shown to have calcium-dependent homophilic binding in the presence of 1 mM Ca²⁺ (Fig. 4, Paper I). Considering earlier results demonstrating that intracellular signaling pattern in hemocytes was changed in the presence of hemolin (Lanz-Mendoza *et al.*, 1996), we hypothesized that soluble hemolin interacts to the hemocyte surface by two mechanisms, directly or indirectly by crosslinking to a receptor or/and Ca²⁺ homophilic interaction with membrane-bound hemolin (Paper I). Later, Bettencourt *et al.* (1999) found Ca²⁺ to be crucial for hemolin binding to hemocytes, using fluorescent microspheres coated with hemolin. They also found that this binding was inhibited by N-acetylglucosamine or N-acetyleneuraminic acid, showing the importance of carbohydrates in this interaction.

Moreover, we have shown that both the *hemolin* gene and the protein are expressed in embryos and neural tissue at different developmental stages.
without bacterial challenge (Fig. 6 and 7, Paper I). The hemolin amino acid sequence is related to the L1 family of neural cell adhesion molecules including Drosophila neuroglian and human L1 and axonin-1 (Fig. 8, Paper I; Ladendorff and Kanost, 1991; Sun et al., 1990). These cell adhesion molecules are expressed in developing neural system. L1 and axonin-1, form homophilic and heterophilic interactions with other cell adhesion molecules, and these interactions are important during the development of the central nervous system (CNS) in vertebrates. L1 and neuroglian consist of six extracellular Ig domains followed by five fibronectin (FN) type III domains linked to a transmembrane domain and a conserved cytoplasmic domain. Similarly, axonin-1 contains six Ig and four FN-III domains, while it is anchored by a GPI-link to the cellular membrane.

Recently, I searched for molecules that are structurally similar (http://cl.sdsc.edu) to hemolin, and found several proteins with Ig-like domains, besides axonin-1, the human poliovirus receptor and human ICAM-1. Interestingly, ICAM-1 is a transmembrane molecule containing five Ig domains and, like hemolin and axonin-1, it is detected as a soluble form in serum (Rothlein et al., 1991). It is normally expressed at very low levels, but it is rapidly up-regulated by cytokine stimulation, enhancing the adhesion of leukocytes to endothelial cells at a site of infection or injury. As described earlier, ICAM-1 is also used as host receptor by various pathogens and parasitized host cells, such as rhinoviruses and the malaria parasite P. falciparum infected erythrocytes. The N-terminal Ig-domain of ICAM-1 is the primary binding site for integrin leukocyte function-associated antigen (LFA-1), mediating adhesion of leukocytes to endothelial cells (Bella et al., 1999). Also this domain interacts with rhinoviruses and P. falciparum infected erythrocytes. Recent studies have revealed that infection with the Autographa californica nuclear polyhedrosis virus (AcNPV) induces hemolin gene expression in H. cecropia pupae (data not shown). Also, the hemolin gene has been cloned from fat body in baculovirus (BmNPV) infected B. mori pupa (Toru Shimada, personal communication). This indicates that hemolin is involved in the defense against viruses. Although the similarity to ICAM-1 is striking, hemolin binding to virus has yet to be shown.

The structure of hemolin has been determined by X-ray crystallography. The four Ig domains (D1-D4) in the molecule are arranged in a horseshoe shape, and D1 interacts with D4, while D2 interacts with D3 (Su et al., 1998). The most N-terminal Ig-domains of axonin-I and L1 share this structure (Freigang et al., 2000; Schurmann et al., 2001). These four Ig domains of axonin-I and neuroglian are required for binding to other CAMs and for mediating cell adhesion, respectively (Fitzli et al., 2000; Rader et al., 1993). Su et al. (1998) proposed that the open structure of hemolin could bind to another horseshoe member by maintaining the same contacts as in the horseshoe fold. However, Schurmann et al. (2001) argue that the horseshoe fold is more
favorable, and suggests that the fully folded horseshoe in axonin-1 mediates the cell adhesion contact. Three-dimensional structure analysis of three N-terminal Ig domains showed that poliovirus receptor binds to virus in an open structure (He et al., 1999). In conclusion, the horseshoe fold of the four Ig domains shared by different IgSF-CAMs might be the basis for the homophilic and heterophilic interaction mediating cell adhesion.

The potential functions of Hemolin
Hemolin’s similarity to insect and vertebrate cell adhesion molecules indicates a regulating role of the adhesion of hemocytes to foreign or own surfaces (Faye and Kanost, 1998). Several observations suggest that hemolin may function as an opsonin or as a pathogen-recognition molecule, with broad specificity, during an immune response. Hemolin is induced by bacterial infection and can attach to bacteria and hemocytes. Also, binding to the surfaces of bacteria and yeast causes the micro-organism to aggregate (Yu and Kanost, 2002). The presence of hemolin increases the ability of M. sexta and H. cecropia hemocytes to phagocytose E. coli cells and yeast cells respectively (Lanz-Mendoza et al., 1996; Zhao and Kanost, 1995). In addition, hemolin changes the hemocyte’s protein kinase C (PKC) activity and the tyrosine phosphorylation pattern (Lanz-Mendoza et al., 1996).

In conclusion, during an infection, hemolin binds to bacterial surfaces, modulates the hemocyte adhesion to pathogens and to own tissue surfaces, and stimulates cellular reactions such as phagocytosis and encapsulation. During development, hemolin is either active in developing tissues or protective against infections.

Hemolin intron enhancer (Paper II)
The expression of the hemolin gene is induced during an infection and is developmentally regulated. To understand the multifaceted control of the gene we wanted to identify the regulatory elements and transcription factors that are involved. The induction of insect immune genes depends on κB-motifs. The upstream sequence and the third intron of the hemolin gene contain putative NF-κB binding sites that were examined (Fig. 1 and 2, Paper II; Lindström-Dinnetz et al., 1995). The intron, but not the 1.5 kb upstream region, was transcriptionally active in Drosophila mbn-2 cells (Fig. 4, Paper II).

This result inspired us to further analyze the involvement of different transcription factors known to regulate mammalian and insect immune genes. For this study we switched to Drosophila SL2 cells earlier demonstrated to be useful for analyzing activators, such as Rel, bZip and HMG1 by co-transfection (Thanos and Maniatis, 1995). The Rel proteins Dif and Dorsal activate a reporter gene construct controlled by the intron (Fig. 5, Paper II). Including the activators C/EBP and HMG1 further enhanced this activity (Fig. 6, Paper II).
The third intron of the hemolin gene contains C/EBP and κB-sites that are separated. It is possible that DNA looping mediates Dif and C/EBP interaction. The architectural protein HMGI may stabilize this interaction, generating synergistic activation of the hemolin gene (Paper II). We conclude that the intron enhancer is used during an infection, since the κB-site is known to be crucial for an immune response. The activity mediated by rat C/EBP and human HMGI with Drosophila Dif demonstrates that these activators are universal. In addition to their enhancing effect, the involvement of these transcription factors could promote specificity in the gene regulation. However, the results do not reflect the high induction achieved upon bacterial injection in pupae, and indicate that the regulation is more complex.

Interestingly, there is an unexamined κB-site situated between the TATA box and the cap site of M. sexta and H. cecropia hemolin genes (Table 1, Paper 4; Zhao and Kanost, 1995). This site was not included in the upstream reporter gene construct analyzed in the Drosophila cells. At that time we decided to use the Drosophila CecAI instead of the hemolin basal promoter. To my knowledge, there have not been any reports describing a functional κB-site in this oddly position.

As described above, ICAM-1 and hemolin proteins share many properties. Interestingly, the ICAM-1 gene expression is induced by cytokines, such as IL-1, TNF-α and IFN-γ. Analysis of the upstream regulatory region of the ICAM-1 gene revealed an activating region containing an NF-κB site and a silencer region (Voraberger et al., 1991). A 1.3 kb upstream region could not confer IFN-γ inducibility in the transfection assay, while a 5 kb upstream region gave a two-fold induction, indicating a responsive element located further upstream. It is possible that the 1.5 kb upstream region of hemolin contains inhibitory regions, and/or that the necessary regulatory elements are situated further upstream or downstream of the transcriptional start site. Interestingly, a new regulatory element (CATTA/T) was found to be an LPS-responsive site necessary for full activation of the B. mori CecB gene (Taniai and Tomita, 2000). Most of the insect immune-responsive genes also contain this motif, including the upstream region of hemolin from H. cecropia and M. sexta.

**Hormone regulation of Hemolin (Paper III)**

Hemolin, gene and protein, is differentially expressed during metamorphosis of the H. cecropia and M. sexta moths (Paper I; Trenczek, 1998; Yu and Kanost, 1999). Increasing titre of the steroid hormone ecdysone causes the insect to molt and to undergo metamorphosis. We were interested to see if the ecdysone active form 20-hydroxyecdysone (20E) could induce hemolin gene expression. And indeed, by injecting 20E into the pupae we could induce the transcription of hemolin in the fat body and in the gut, analyzed by Northern blot (Fig. 1, Paper III). Overall, a similar response was achieved in the gut as in the fat body (Fig. 4, Paper III). In order to determine whether the 20E receptor complex directly
activates the gene, we co-injected the protein-synthesis blocking reagent cycloheximide with 20E. Preliminary data showed that the hemolin gene expression was inhibited, indicating an indirect effect of 20E (Fig. 2, Paper III).

We then wanted to analyze the effect that an infection has on hemolin gene regulation during development. By pre-injection of 20E before a bacterial challenge we showed that the hormone enhances the expression of several immune genes analyzed by Northern blot. Hemolin, as well as attacin A, lysozyme, and cecropin B gene expression was enhanced (Fig. 3 and 4, Paper III). These results indicate that more hemolin and antibacterial peptides are needed to encounter an infection during development.

By comparing the upstream regions of M. sexta and H. cecropia I found three novel regions, which were named HemI, HemII and HemIII. They all bind specifically to nuclear proteins as demonstrated by EMSA (Fig. 5, Paper IV). HemI and HemIII contain sequences similar to hormone responsive element (HRE) and HemII shows similarities with interferon regulatory factor (IRF-1 and IRF-2) binding element (IRFE) (Table 1, Paper IV). We will further analyze these regulatory elements in hemolin upstream region in response to hormone using transfection assays in 20E responsive M. sexta GV-1 cells. Our current hypothesis is that 20E induces a hormone receptor that subsequently induces the hemolin gene by binding to HemI and/or HemIII. We will also investigate the response to dsRNA and viruses, since HemII is similar to IRFE.

In agreement with our result it has been demonstrated that hemolin from the gypsy moth Lymantria dispar is up-regulated by 20E (Lee et al., 2002). It is up-regulated when first instar larvae are entering diapause. Unlike H. cecropia, L. dispar enters hibernation as a pharate first instar larva instead of a pupa. The hemolin induction is dependent on ecdysteroids, being inhibited by KK-42, an imidazole derivate that inhibits ecdysteroid biosynthesis.

Recently, a hemolin RNA interference (RNAi) experiment was completed. Female pupae injected with ds hemolin developed into adults, but the embryonic development was affected in the next generation (Bettencourt et al., 2002). The exact target for this gene silencing effect is not known. The injected ds hemolin may silence the expression of hemolin in the ovary, disturbing embryo development. Another explanation is that the pupae carry latent viral infections and without hemolin the embryo cannot survive.

To conclude, the moths need hemolin during metamorphosis but it is not clear what role(s) hemolin has, it may be used for protection and/or take part in the development of new tissues.

Hemolin meets Yippee (Paper IV)
In order to use Drosophila genetics for studying hemolin function and regulation, several attempts have been made to clone a putative hemolin homologue in Drosophila. I tried to amplify the corresponding gene with degenerated primers in PCR reactions using Drosophila cDNA libraries and
total RNA as template without success. I then performed a two-hybrid screen using *H. cecropia* hemolin as bait, searching for interacting proteins encoded in a *Drosophila* cDNA library (prey). Since hemolin is known to form homodimers (Paper I), the idea was that its putative orthologue in *Drosophila* would be found by its dimerization to hemolin. If not, any hemolin interacting protein from *Drosophila* could be used as possible baits to find the hemolin orthologue. At the same time, it was of interest to reveal what kind of proteins hemolin interacts with.

Using hemolin as a bait, 16 clones were isolated that interact specifically with hemolin. 15 of these clones encoded the same protein. In my excitement, I jumped around shouting “Yippee! Yippee!”, and this is how this new protein received its name. Database searches revealed a novel, highly conserved eukaryote gene family distantly related to Zn-finger proteins (Fig. 3, Paper IV). It appears to be a 13.7 kDa cytoplasmic protein with conserved cysteine residues. The cysteine residues might bind a metal ion that establishes the folding of this small protein. A computer analysis of the predicted 3D structure was performed and revealed a globular domain (data not shown). The hemolin interaction to yippee was specific, and a truncated form of hemolin containing the signal peptide and the first Ig-like domain significantly bound to yippee, although at a lower level (Table 1, Paper IV).

Do hemolin and yippee interact *in vivo*? We found that in the yeast cell yippee clearly binds to hemolin. Predicted to be an intracellular protein, yippee could take part in the folding and secretion of secretory proteins like hemolin. Hemolin is not as ubiquitously present in eukaryotes as yippee, but still, other intracellular proteins could share the interaction epitope. The importance of the interaction between hemolin and yippee needs to be determined in *H. cecropia*.

I also cloned and sequenced the human *yippee* gene and it showed 76% identity to *Drosophila* yippee at the amino acid level (Paper IV). In different human fetal tissues two *yippee* gene transcripts were detected and were expressed in different relative proportions (Fig. 8, Paper IV). For example: the level of the expression in the heart was the same, while the brain contained 4.4 times more of the 2.4 kb transcript than the 1.4 kb transcript. In *Drosophila* one 1.2 kb transcript was detected in RNA prepared from mbn-2 cells, SL2 cells, and from whole larvae, pupae or adults (Fig. 7, Paper IV).

Database searches revealed yippee-related genes from diverse species including *Drosophila* and human, and these genes was regarded as yippee paralogues (Table 2 and Fig. 4, Paper IV). Interestingly, in a recent database search the human yippee paralogues, Human II and *Drosophila* II, showed 96% and 88% identity respectively, to the DiGeorge syndrome-related protein FKSG4 (AF305195) from humans. DiGeorge syndrome is a common innate disorder characterized by neural-crest-related developmental defects (Epstein, 2001).
Yippee as bait
To reach a better understanding of the function of yippee, we wanted to identify yippee interacting proteins and at the same time try to clone the *Drosophila* homologue of hemolin. Using the interaction trap system, I used yippee as bait screening the *Drosophila* disc cDNA-library. Ninety-six clones were re-analyzed for their ability to grow on Leu⁻ plates and give blue color on X-gal plates. Twenty-five prey plasmids were isolated and 10 were re-transformed to analyze the specificity of the interaction (Table 1). I then sequenced these and checked for similarity in the databases (Table 1). Yippee interacted with diverse kinds of proteins with no common character. It interacted with secretory proteins, with proteins involved in different degradation pathways and with proteins involved in biosynthesis. However, the *Drosophila* homologue of hemolin was not found among these 10 clones.

The interaction having the highest β-galactosidase value was with the yippee-interacting protein 1, Yip1, which showed 68% identity to the *Drosophila* gene spen (split end). Spen encodes a large protein (600 kDa) involved in glia cell migration and axon guidance (Kuang *et al.*, 2000). It is localized to the nucleus and contains an RNA-binding region, RNP-1. Yip1 and spen seem to have different polyadenylation sites, indicating that Yip1 might be a shorter spliced variant of spen.

Analysis of the Yip2 sequence revealed a protein containing a thiolase domain and indicated homology to acetyl-coenzyme A acetyltransferase. Thiolases take part in the last step of the degradation of fatty acids in the peroxisome.

Yippee also interacts with proteins taking part in the degradation of proteins inside and outside of the cell. Yip5 is identified as Rpn11 in FlyBase ([http://flybase.bio.indiana](http://flybase.bio.indiana)). Rpn11 is one subunit of the regulatory complexes (RC) in the *Drosophila* 26S proteasomes involved in degradation of multiubiquitin proteins (Holzl *et al.*, 2000). The RC serves to recognize these proteins and prepare them for degradation in the 20S proteolytic complex. Moreover, Yip3 showed similarity to the 20S proteasome β2 subunit in *Drosophila* and is reported to contain the multispecific protease domain of the proteasome ([http://flybase.bio.indiana](http://flybase.bio.indiana)). It is possible that yippee targets missfolded proteins for degradation and in the interaction trap screen recognizes hemolin as a misfolded protein. In addition, Yip7 encodes a serine-type endopeptidase and shows similarities to chymotrypsin, which normally takes part in the degradation of intestinal proteins. Although achieving the weakest interaction with hemolin detected by β-galactosidase activity, two out of 10 independent clones containing different sizes of insert were isolated.
Table 1. Description of Yippee interacting proteins (dmYip) 1-9. Homologous proteins found in database searches and their amino acid (aa) identity to dmYip 1-9, and the interaction between *Drosophila* Yippee and dmYip 1-9.

<table>
<thead>
<tr>
<th>dmYip Accession number</th>
<th>Database definition</th>
<th>Accession number</th>
<th>Species</th>
<th>Identity (%)</th>
<th>Overlap (aa)</th>
<th>Interaction with dmYippee β-galactosidase activity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 AF195186</td>
<td>Split ends (spen)</td>
<td>AF188205</td>
<td><em>Drosophila</em></td>
<td>68</td>
<td>51</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>2 AF195187</td>
<td>Acetyl-Coenzyme A acetyltransferase 2</td>
<td>AAH01918</td>
<td>Human</td>
<td>56</td>
<td>376</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>3 AF195188</td>
<td>20S proteosome β2 subunit</td>
<td>AF025791</td>
<td><em>Drosophila</em></td>
<td>27</td>
<td>140</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>4 AF195185</td>
<td>No match</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>5 AF195189</td>
<td>Rpn11 (proteasome endopeptidase)</td>
<td>AF145313</td>
<td><em>Drosophila</em></td>
<td>96</td>
<td>229</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>6 AF195190</td>
<td>Ribosomal protein L5</td>
<td>AF008229</td>
<td><em>B. mori</em></td>
<td>77</td>
<td>139</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>7 AF195191</td>
<td>Chymotrysin</td>
<td>U03760</td>
<td><em>L. cuprina</em></td>
<td>54</td>
<td>122</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>8 -</td>
<td>Elongation factor 1α</td>
<td>X06869</td>
<td><em>Drosophila</em></td>
<td>94</td>
<td>79</td>
<td><img src="#" alt="Graph" /></td>
</tr>
<tr>
<td>9 -</td>
<td>Mitochondrial 16s rRNA</td>
<td>X53406</td>
<td><em>Drosophila</em></td>
<td>68</td>
<td>258</td>
<td><img src="#" alt="Graph" /></td>
</tr>
</tbody>
</table>
*Drosophila* yippee interacts with another type of protein, Yip6, which encodes a putative structural component of the ribosome, the ribosomal protein L5. L5 binds to 5S rRNA in the ribosome, but other interactions have been reported. L5 interacts with the oncoprotein MDM2, a RING finger protein, and to p53, known to be involved in apoptosis. In addition, yippee picked up clones pB42-20 and pB43-20 with sequence similarity to mitochondria 16S rRNA and elongation factor 1α (EF1α), respectively. EF1α is involved in protein biosynthesis, and catalyzes the targeting of tRNAs to the ribosome. The isolation of rRNA from a poly-T primed cDNA library was unexpected, but this interaction suggests that yippee associates with RNA and/or RNA binding proteins. The fact that yippee is distantly related to Zn-fingers known to bind DNA, and interacts with spen, containing RNA binding domains, and proteins involved in translation of mRNA, suggests that yippee may be part of a RNA-protein complex. Another interesting correlation is that yippee interacts with spen, which is involved in glia cell migration and axon guidance in *Drosophila*, and that the human yippee paralogoue was related to the DiGeorge syndrome-related protein, and thus probably involved in neural-crest-related developmental defects.

Finally, the *yippee* gene is highly conserved in eukaryotes indicating that this protein is of general importance in eukaryotic organisms.

**Specificity in immune response (Paper V)**

*Drosophila* feeds on decaying fruit and is constantly exposed to microbes. The immune responses of the insect have been best characterized upon bacterial infections and to some extent fungal infection. However, in these studies the bacteria were injected or introduced by pricking with a needle dipped into bacteria into the hemocoele. It has been shown that the immune response in *Drosophila* possesses alternative pathways, depending on the infecting bacteria or fungi (Lemaitre et al., 1996). Specific pattern recognition molecules have been isolated and specific effectors molecules are induced. We wanted to extend this knowledge of specificity by including parasites and viruses and comparing the immune response against *Drosophila* naturally infecting microbes in a normal way of infection, i.e. by feeding. The following microbes were used to infect *Drosophila* flies: *Drosophila* C virus, a single-stranded RNA+ Picornavirus that enters the gut heading for the fat body (Gravot et al., 2000); *Serratia marcescens* Db11, a non-pathogenic strain of Gram-negative bacteria (Flyg et al., 1980; Flyg et al., 1983); *Beauveria bassiana*, a fungus that penetrates the cuticle and kills the flies in days (Lemaitre et al., 1997); and *Octosporea muscadomesticae*, a parasitic protozoa that penetrates the gut wall (Smallridge et al., 1995).

The flies were fed for 24 h on a sugar solution and then total RNA was prepared from whole flies. The flies infected with *Beauveria* were shaken in the fungal spores prior to feeding. The gene expression pattern was investigated
on *Drosophila* Affymetrix GeneChip representing almost 14,000 *Drosophila* genes.

We selected those genes that were increased by comparing the gene expression profiles from the different infections with normal flies. The microbial organisms generated different strengths of immune responses: the *Beauveria* infection generated the strongest response with 298 genes induced, the *Octosporea* infection induced 127 genes, while the DCV and *Serratia* infection induced least genes, 11 and 10 respectively.

By comparing the induced genes among the different infections, several uniquely expressed genes were identified, demonstrating the specificity of the immune response (Fig. 3, Paper V). In all four treatments, several serine proteases were induced, and several of these were unique (Table 2, Paper V). The serine proteases are important in initiating diverse immune functions. Another interesting result was that *Octosporea* induced several *lysozymal* genes, earlier regarded as being constitutively expressed in the *Drosophila* gut (Table 1, Paper V). The major group among the unique genes had no assigned function and these genes are of particular interest for further characterization (Fig. 2, Paper V).
All types of organism require highly controlled systems in order to develop, maintain and protect themselves against pathogens. These processes are dependent on the co-operative action of molecules and cells, and on the presence and amount of the molecules regulating the specificity of the action. The complete genetic information of several organisms is known, but we are just at the beginning of understanding how the RNA and proteins function together to make up an organism. Using insects as a model provides the tools to investigate development and innate immunity.

Several researchers before me were intrigued to work with hemolin, this Ig-like molecule that is a major inducible protein after bacterial challenge. Today, other proteins have been found in both mammals and insects to have nearly identical tertiary and quaternary structures as hemolin, even though the amino acid sequence similarity is low. The functional relevance of these protein structures might also be conserved and this hypothesis encourages further characterization of hemolin.

This thesis work has demonstrated:
1). That hemolin possesses cell adhesion properties. Hemolin exhibits calcium-dependent homophilic binding properties and a 52 kDa form of hemolin is associated to hemocyte membranes.
2). That hemolin shares different features with cell adhesion molecules of the IgSF: the vertebrate axonin-1, involved in the development of neural tissues, and to human ICAM-1, promoting adhesion between endothelial and leukocyte cells at a site of injury.
3). That hemolin is developmentally regulated in H. cecropia. The hemolin gene is expressed in embryos and pupae neural tissues. It is also induced in the fat body of pupae, by 20E injections. Preliminary, this induction is indirect through a 20E-induced transcription factor. Also, injecting 20E and bacteria into the pupae enhanced the gene expression of immune genes including hemolin.
4). That the third intron of the hemolin gene contains an enhancer. The Rel factor Cif can bind to a κB-motif in this intron. The activators Dif, C/EBP and HMGI synergistically transactivate reporter gene constructs through the intron in SL2 cells.
5). That the Drosophila and human yippee genes are members of a novel gene family highly conserved in eukaryotes. The yippee gene is expressed in different Drosophila developmental stages and in different human tissues, suggesting that yippee is ubiquitously expressed in all types of cells. Yippee interacts with diverse kinds of proteins analyzed by the two-hybrid system in yeast. Also, that yippee related genes exist in diverse organisms.
6). That *Drosophila* responds specifically to different naturally infecting microbes by inducing unique sets of genes.

**Future perspectives**

We show that the regulation of the *hemolin* gene is complex and reflects the multifaceted induction of hemolin (Paper II and III). In order to further characterize the regulation of the *hemolin* gene, several methods could be used. First, by DNase I footprint, locate the areas responsible for bacterial induction and/or hormone regulation. Secondly, interesting regions could be further analyzed in transfection assays by using mutations and deletions constructs in response to LPS and ecdysone. It would be interesting to combine the intron and the upstream region together with the factors C/EBP, HMGHI and the steroid hormone.

Recent developments in germline transformation with transposon vectors allow investigation of gene function in non-*Drosophilid* insects. Uhlirova et al. (2002), demonstrated for the first time a heat-inducible transgenic expression in *B. mori*. By using the transposon piggyBac, a versatile vector for insect transformation, a transgene under the control of *Drosophila* heat shock promoter hsp70 was inserted into the *B. mori* genome. The expression of the transgene was induced by heat. It would be exciting, though challenging, to use this technique to further analyze hemolin function and regulation in the moth *B. mori*. The hemolin functional role could be examined by overexpressing the hemolin protein or by knocking the *hemolin* gene by inducing the production of double-stranded RNA and studying the phenotype. In addition, important regulatory elements for hemolin expression during development and infection could be determined. In these experiments piggyBac could be used to introduce reporter gene constructs under the control of different regulatory elements.

With the help of hemolin, I discovered a new highly conserved gene family, yippee, with so far unknown function (Paper IV). There are several good models to further study the function of yippee. The yeast open reading frame, sharing sequence similarities to yippee-related proteins, is not essential under normal growth conditions. It would be interesting to analyze this knockout in comparison to the wild type, by separating the proteins in a two-dimension gel and identifying the influenced proteins by mass spectroscopy. Another possibility is to silence the *yippee* transcript by using RNA interference in *C. elegans*, which has been successfully used for other RNAi studies, and comparing it with wild type as above. Interestingly, there is a *Drosophila* deficiency (Df(1)C246) available with deletions in the 11D-12A region at the X chromosome where the *yippee* gene was mapped (Paper IV). I will follow, with great interest, the progress determining the role that DiGeorge syndrome-related proteins have in this nerve-crest disorder.
In the last part of my thesis, I turned my focus to the specificity of the *Drosophila* innate immune response (Paper V). We revealed several genes that were specifically induced in response to the parasite *Octosporea* when naturally infected. Among these specific genes, we will search for possible effector molecules that can be used against pathogens for humans, especially for the malaria parasite *P. falciparum*. The malaria parasite can survive in *Drosophila* when injected, but it is killed when fed, indicating that there are molecules expressed in the gut or that the gut structure stops the parasite infection (Schneider and Shahabuddin, 2000).
ACKNOWLEDGEMENTS

Many people have contributed to my work with support of many kinds.
I first want to thank my supervisor, Ingrid Faye, for your optimism and
encouragement, for giving me leeway in pursuing my research interests, and for
being a good friend. I have enjoyed being a member of your research group
which, among other things, has given me the opportunity to meet fascinating
people from all over the world, including the Azores, Eritrea, Mexico, China,
France, Peru, Poland, Japan, Italy, USA, Island, and India. I am also very
grateful to Elisabeth Haggård-Ljungquist, for your commitment to my research,
and for always keeping your door open for me; Leif Isaksson, for accepting me
as a PhD student some time ago…-- no more Kattis 0.5p at the staff meetings;
Håkan Steiner, Ylva Engström and Dan Hultmark for your dedication, and for
being such great sources of knowledge; Ingrid Lindström-Dinnetz, Christina
Thyle’n, Vanessa Dalton and George Farrants, for whom I owe a special thanks
for their editorial help.

I also want to express my deep gratitude to past and present lab colleagues:
Ingrid for your friendship, and for all the laughs and tears we shared during my
first years; Sun, for teaching me the secrets of purification -- even though it
proved to be a very freezing experience… Atchoo!; Raul, for showing me that
science can be pursued without protocols and in the spirit of salsa music --“I
squeezed the egg with my thumb like this…”; Humberto, for always being so
cheerful and happy -- “And now welcome to the must famous journal club… I
“surbive”; Yohannes, for being such a gentleman and for all our interesting talks
about Eritrea, our respective children, how to keep cell lines etc.; Olle, for all the
stimulating discussions on science and life in general, for your constant
assistance and our great co-work. Lee and Makoto, for sharing all your
postdoctoral knowledge and wisdom; Katarzyna, Zongpei, Isabel and Christine,
for doing excellent diploma work. Finally, Jenny and Katarzyna, I wish you
both, good luck!

Thanks to all the staff of the genetics department for being such amusing and
wonderful people to work with; Sara and Jesper E, --“one night in Paris”;
Richard, --I believe that I won the last strong man/woman contest; Tao, the
cheerful two-hybrid associate, Alex, the traveler with great fighting spirit;
Anders, Mr. knowledge in trees; Clara, the cool bicycle rider; Petri, the
marathon man; Joakim, “you humors gay”; Annette, a girl classical; Magnus, the
newly become daddy, congratulations! Irja, -- the flies and I miss you; Virginia,
--I miss you too; Latif, my best colleague; Gunnel, the terrific song writer;
Björn, the man making my life with my computer almost agreeable; Christina, --
thanks for your concern; Agneta, -- I will not forget Mendel; Hans, -- I will not
forget “kanelbullarna”; Santanou, -- I always feel so happy when I see you; Ingrid Bergh, -- thanks for the tetrad-analyzis days; Jesper T, thanks for your entertaining social visits to our room and for making me remember Tibet.

To the present and former participants of the Every Second Monday Innate Meetings: special thanks to Ulla-Maja, for all your useful comments on my project, and for being a great travel companion to “Islandur” and USA; Gunnel for sharing your great scientific knowledge, and for making me jealous of your adventure trips.

To the microbes: thank you Daiwu, for the instructive PCR and cloning discussions; Anne-Marie and Magnus, for enlightening me about your spider friend Tarantella; Astrid, for your corridor singing; Mats, for the d2jmts; Lena, for your support and the litre of Cif; Anita Boman for letting me use “snurran” to prepare nuclear extracts; Inga-britt, Helena, Hasse, Marianne, Margareta, Monica, Veronica, Ann, Salim, Jonas, Asgier, and Krusse for all the fun moments we have had in the coffee-corner.

A big “thank you” to my dear friends outside the lab: Per and Carina, for our relaxing sailing and skiing holidays; Krister and Malin, for caring, and for making life with two pre-school children seem like “a walk in a ball park”; Lena, Mats, L., Therese and Mats, for all the great moments we have had together with our children; my dancing friend, Lotta for being a great friend -- can you believe we performed in those “not-quite see-through outfits?”…paderbore, paderbore, kick boll change, double-turn pirouettes.

My deepest gratitude to my wonderful family: Marie, Tomas, Sara and Hanna, Inger and Lennart, for including me in your family, and for providing such a loving and peaceful environment; the Åland Sport Fishing Gang, which includes Christer, Maria, Johan, Gustav, Mikael, Marie, Philip, Linn and Christian, for your deep commitment to fish preservation; Tomas, Sarah, Cecilia and Christian, for your deep care and love. Pappa and Margareta, for being so supportive, for giving Erik and me time together, and for my new associates Dell and Armada E500; Mamma, for being so happy about the “yellow color in the test-tube” and for always believing in me; Bror, for occupying a big place in my heart and for bringing Vanessa into our family.

Finally, I am so grateful to my own little family, the LindRoxs: Erik, thank you for being a wonderful team member, for the fun we have had and will have together – both above and under water – for your unwavering support and love, and for so many other things; Linnea and Emil, for giving me such a rich life with lots of “bus” and laughs. I am very grateful for sharing my life with you, and I love you so very much.
Biologiskt liv brukar definieras som avgränsande system vilka har förmåga att ta upp ämnen från omgivningen, använda dessa till att föröka sig samt framställa energi. Med denna definition framstår cellen som livets grundenhet. Organismer som bakterier och jästsvampar består av endast en cell som ensamt klarar av organismens alla funktioner, medan högre organismer är flercelliga och uppbyggda av många olika celltyper som specialiserat sig på skilda uppgifter och funktioner.

Celler i flercelliga organismer får inte konkurrera med varandra om livsutrymmet utan måste samarbeta. Detta kräver omfattande kommunikations och regleringssystem. Om cellen förlorar denna kontroll får den en fördel jämfört med andra celler vilket kan ge upphov till störningar av olika slag. Encelliga och flercelliga organismer behöver även skydda sig mot patogener. Vi utsätts ständigt för mikroorganismer (mikrober) och många olika typer av immunförsvar har utvecklats.

**Hur blir vi sjuka av mikrober?**
Patogener orsakar skada på olika sätt. Dom kan producera giftiga ämnen (toxiner, ämnesomsättningstoner, eller enzymer) som utsöndras under tillväxt eller frigörs när patogener dör. Vissa sjukdomar orsakas mer av organismens immunreaktion än på själva smittämnet som i överkänslighetssjukdomar (allergi) och i en del infektionssjukdomar, t.ex. tuberkulos. Immunsytemet kan även bli överaktiverat och vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; vissa färger som blodet; viss...

**Vad gör vi för att försvara oss?**

Vår hud är ett tjockt lager av celler som skyddar kroppen från påverkan utifrån som stötar, värme och kyla och även mot t.ex. bakterier. De flesta bakterier kommer in i våran kropp via maten till magen, via luften till lungorna eller genom ett sår på huden. I magen möter bakterierna en sur miljö och nedbrytande enzymer vilket inte gynnar deras tillväxt. De som överlever detta får möta vårt immunförsvar.


**Insekter som modellorganism**


För 10 år sedan fann man i Ingrid Fayas grupp indikationer på hur det medfödda immunförsvarset styrs, vilket gav grunden till ett nytt forskningsområde. Regleringen av immun-generna skedde via Rel transkriptionsfaktorer (Rel/NF-κB) och visade sig vara besläktat med regleringen i människan och aktiverar samma medfödda immunförsvar (Fig. A).

![Fig. A. Från infektion till försvar. När en *H. cecropia* puppa blir infekterad utav bakterier så aktiveras en rad händelser som leder till bl.a. produktion utav antibakteriella peptider. Proteiner utanför cellen känner igen bakterien vilket leder till att cellen blir aktiverad. Signalen förs vidare inuti cellen och resulterar i aktivering av Cif, ett Rel-protein. Cif kan nu gå in i kärnan och binda till ett DNA element framför immune generna vilka nu transkriberas och immun protein bildas t.ex. Cecropin. Dessa utsöndras från cellen och kan döda bakterierna.]
Min forskning
Jag är fascinerad över kommunikationen mellan celler och regleringen utav cellernas aktivitet, vilket utgör grunden för en organism’s immunförsvar och utveckling.

De modellorganismer jag har använt i mina studier är silkesfjärilen *Hyalophora cecropia* och bananflugan *Drosophila melanogaster*. Jag har fokuserat mig på att studera Hemolins funktion och genereglering under *H. cecropia* utveckling och under immunförsvarset.


För *Drosophila* har det utvecklas många kraftfulla tekniker och för att enklare kunna undersöka vilken funktion Hemolin har så ville vi hitta motsvarande protein i flugan. Vi fann inte Hemolin utan ett nytt protein som vi döpte till Yippee. Det visade sig att Yippee var väldigt konserverat och finns i alla djur och växter, även i människan. Troligtvist används Yippee proteinet inuti cellen.

Tills sist undersöker vi för närvarande hur specifikt det medfödda immunförsvarset är. Vi matar *Drosophila* flugor med olika typer av mikrober och jämför vilka gener som specifikt är påslagna.

Med dessa ord så välkomnar jag er in i min forsknings värld… men först en liten grundkurs på nästa sida.

*H. cecropia* /Linnea
Grundkurs
Människans omkring 30 000 gener är kartlagda vilket är det största forskningsprojektet i biologins historia och som tog 10 år att utföra (Hugo projektet och Celera). I cellerna ligger arvsanlagen inkodade i kromosomernas DNA. Alla celler i en vuxen människa innehåller en total uppsättning av arvsanlaget. I arvsanlagen finns den information som bestämmer hur de olika proteinerna skall konstrueras (Fig. B) och när det skall tillverkas, vilket i sin tur styr cellens funktion. Men eftersom cellerna är specialiserade på en bestämd uppgift behöver de inte använda alla gener. Efter kartläggningen utav generna kvarstår dock det riktigt stora arbetet att tolka generna och förstå proteinernas funktion. Citat från Craig Venter: “Liljan har 90 miljoner baspar, människan har bara 3 miljoner. Jag tror inte att de är smartare än vi för det. Men det är ändå de som får sista ordet eftersom de läggs på våra gravar.”

REFERENCES


tingvall, t. o., roos, e. and engström, y. (2001 a) the gata factor serpent is required for the onset of the humoral immune response in drosophila embryos. proc. natl. acad. sci. usa 98: 3884-8.


tzou, p., reichhart, j. m. and lemaître, b. (2002) constitutive expression of a single antimicrobial peptide can restore wild-type resistance to infection in immunodeficient drosophila mutants. proc. natl. acad. sci. usa 99: 2152-7.

uhlirrova, m., asahina, m., riddiford, l. m. and jindra, m. (2002) heat-inducible transgenic expression in the silkmoth bombyx mori. dev. genes. evol. 212: 145-51.

underhill, d. m. and ozinsky, a. (2002) toll-like receptors: key mediators of microbe detection. curr opin immunol. 14: 103-110.

wang, y., willott, e. and kanost, m. r. (1995) organization and expression of the hemolin gene, a member of the immunoglobulin superfamily in an insect, manduca sexta. insect molecular biology 4: 113-123.

werner, t., liu, g., kang, d., ekengren, s., steiner, h. and hultmark, d. (2000) a family of peptidoglycan recognition proteins in the fruit fly drosophila melanogaster. proc. natl. acad. sci. usa 97: 13772-7.

voraberger, g., schafer, r. and stratowa, c. (1991) cloning of the human gene for intercellular adhesion molecule 1 and analysis of its 5' regulatory


