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*Minireview*

# The humoral antibacterial response of *Drosophila*

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*Drosophila*, like other insects, responds to the injection of bacteria by the rapid and transient synthesis of a battery of potent antibacterial peptides. Only a few of these peptides have been fully characterized to date. We review our recent data on the control of the expression of a gene encoding one of the induced peptides, i.e. diptericin. Our data highlight the role of proximal cis-regulatory motifs similar to regulatory elements binding NF-κB and NF-IL6 in promoters of some immune genes of mammals. We argue that the *Drosophila* host defense is homologous to the mammalian acute phase response.

Insect immunity; *Drosophila*; Antibacterial peptide; Acute phase; NF-κB; NF-IL6

## 1. INTRODUCTION

Insects have long been known to be particularly resistant to bacteria. While early workers in the late 19th century attributed this resistance to phagocytosis and to encapsulation by hemocytes [1,2], several independent studies established around 1920 that insects could be protected against the injection of lethal doses of bacteria by previous administration of low doses [3–5]. This induced protection was correlated to the appearance of a potent antibacterial activity in the cell-free hemolymph. The early studies, and most of the subsequent investigations in the field of insect immunity, were performed on large-sized insect species. It was only in 1972 that the problem of the inducible antibacterial activity was directly addressed in the small-sized *Drosophila*. Studies by Boman and associates [6] demonstrated that in *Drosophila*, like in other insect species, a primary infection can induce a protection against a secndary infection which otherwise would be lethal. However, as Boman later put it, 'the biochemistry behind this phenomenon could not be worked out at that time' [7]. In spite of the obvious interest of *Drosophila* as a model system, Boman and associates turned to the large pupae of the *Cecropia* moth for the first successful isolation of induced antibacterial molecules (cecropins [8] and attacins [9]), while other groups worked on large-sized fly species, like *Sarcophaga peregrina* (isolation of the cecropin-like sarcotoxins I [10], the attacin-related sarcotoxins II [11] and sapecin, a homologue of insect defensin [12]) or *Phormia terranovae* (isolation of diptericins [13] and insect defensins [14]) (for reviews on these peptides, see e.g. [15–17]).

In the mid-eighties, two independent studies confirmed that *Drosophila*, like other insects, responds to the inoculation of bacteria within a few hours by the de novo synthesis of several peptidic or polypeptidic molecules, some of which were presumed to be homologous of cecropins and attacins [7,18]. However, still at that time, the structures of the molecules remained elusive, as a consequence of the low amounts which could be extracted from these small-sized insects.

## 2. THE INDUCIBLE ANTIBACTERIAL PEPTIDES OF *Drosophila*

The first information on the sequences of inducible antibacterial peptides of *Drosophila* was obtained from DNA cloning studies published in 1990. Using a cDNA clone corresponding to the major cecropin isolated by Natori's group from *Sarcophaga peregrina* [10], Hultmark and associates [19,20] were able to characterize in the genome of *Drosophila* a compact cluster comprising three expressed cecropin genes plus two pseudogenes. Two of these genes ($A_1$, $A_2$) code for a cecropin with a deduced amino-acid sequence identical to the major cecropin of *Sarcophaga peregrina*. The other (B) of the three expressed genes codes for a cecropin which differs by five conservative amino-acid replacements. In association with Hultmark's group, we isolated in 1990

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CECROPIN A1/A2 : GWLKKIGKKI ERVGQHTRDA TIQGLGIAQQ AANVAATAR
CECROPIN B :     GWLRKLGKKI ERIGQHTRDA SIQVLGIAQQ AANVAATAR
CECROPIN C :     GWLKKLGKRI ERIGQHTRDA TIQGLGIAQQ AANVAATAR

DIPTERICIN :  DDMTMKPTPP PQYPLNLQGG GGGQSGDGFG FAVQGHQKVW
              TSDNGRHEIG LNGGYGQHLG GPYGNSEPSW KVGSTYTYRF PNF
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Fig. 1. Structures of inducible antibacterial peptides of *Drosophila*. The amino-acid sequences of cecropins [19,20,23] and diptericin [21] were deduced from DNA cloning studies; cecropin A was directly isolated from immune adults of *Drosophila* in our laboratory and the deduced sequence [19] was confirmed by Edman degradation and molecular mass determination ($m/z$ = 4155.2). Bold residues differ among the three expressed cecropins [19,20,23]. We suspect diptericin to be post translationally modified by O-glycosylation, as demonstrated in the case of diptericin from the fleshfly *Phormia terranovae* (unpublished).

a *Drosophila* cDNA encoding a novel member of the diptericin family of inducible antibacterial peptides [21]. We used an oligonucleotide probe corresponding to the peptide sequence of the newly-isolated diptericin from the fleshfly *Phormia terranovae* [22]. A fourth cecropin gene (C), encoding a peptide which differs from cecropin A by three conservative amino-acid replacements, was later described [23]. In essence, these studies established that *Drosophila* expresses, in response to bacterial challenge, genes encoding antibacterial peptides which are homologous to cecropins and diptericins, previously isolated from other insect species (Fig. 1).

Taking advantage of the recent refinements in the methods of analytical chemistry, we have started the direct isolation and characterization of the antibacterial peptides of immunized *Drosophila*. An exciting first result was that a major inducible peptide is a novel 19-residue, proline-rich cationic peptide which carries a substitution which is mandatory for full biological activity of this molecule, which we have named drosocin [24]. Drosocin is active both against Gram-positive and Gram-negative bacteria. We have also identified a peptide corresponding to the sequence deduced by the Hultmark group from cecropin genes $A_1$ and $A_2$ (Fig. 1) and a novel member of the family of insect defensins. The sequence of *Drosophila* defensin and that of its gene will be reported in detail elsewhere [25].

The isolation of other inducible immune peptides of *Drosophila* is in progress and to date we have characterized half a dozen of novel cationic, small-sized (2 to 9 kDa) antibacterial peptides (unpublished). *Drosophila* also produces larger-sized antibacterial polypeptides, according to the data of earlier studies [7,18], and we speculate that the total number of inducible antibacterial molecules is well in excess of a dozen. It is at present unclear why *Drosophila* would need such a large number of different antibacterial peptides for its protection. A possibility is that the activity spectra of the various peptides are complementary and allow the insect to respond to a large variety of invaders. It may also be that some of these molecules synergize in a way which we still do not understand.

## 3. EXPRESSION OF THE IMMUNE GENES OF *Drosophila*

Studies performed on the expression profiles of the genes encoding cecropins [19,20,23], diptericin [21], defensin [25] and drosocin [24] in bacteria-challenged *Drosophila* yielded results similar to those obtained in other species of the recent insect orders (namely in *Hyalophora cecropia* (reviewed in [15]), *Manduca sexta* [26], *Sarcophaga peregrina* [11,27–29], *Phormia terranovae* [30]). The genes are rapidly transcribed following challenge (1 to 2 h), the transcription rate increases over a period varying between 6 and 24 h, depending on the gene, and thereafter either stops or levels off. Not all the genes are transcribed with the same intensity during the response; the induction of the drosocin gene for instance is considerably stronger than that of defensin in larvae and adults of *Drosophila* [24,25]. Some of the genes are preferentially transcribed in challenged pupae: this is the case for the cecropin B and C genes, for instance, whose induced transcriptional rate is negligible in larvae and adults when compared to that of the cecropin $A_1$ and $A_2$ genes [23]. Interestingly, the expression of some of these genes can occur in the absence of an apparent immune challenge: the defensin gene for instance is expressed in normal early pupae [25] (cf. also [29] for *Sarcophaga*). The fat body, a functional equivalent of the mammalian liver, is a major site of transcription of the inducible antibacterial peptides; blood cells also participate in the production of these molecules and for some of the peptides, other tissues may be involved (e.g. pupal hindgut for cecropin C [23]; gut for defensin [25]).

The humoral antibacterial response of *Drosophila*, like that of other insects, is strongly evocative of the acute phase response of mammals (reviewed in [31]); in contrast it does not exhibit the hallmarks of the mammalian lymphocyte response, i.e. specificity and memory. The acute phase response is well preserved throughout phylogeny and plays important roles in the host defense against tissue damage and infection. In mammals, this response is induced by a variety of stimuli such as bacterial infections or tissue injury and is characterized by significant alterations in the serum

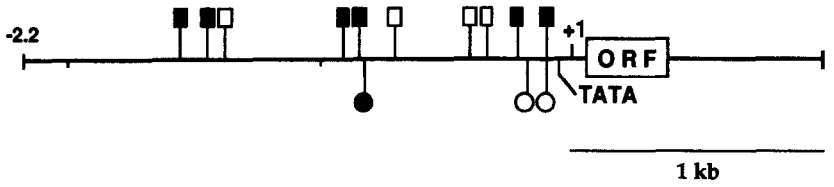


Fig. 2. Promoter elements of the *Drosophila* diptericin gene. Distribution of sequences homologous to vertebrate immune gene response elements in the 2.2 kb diptericin promoter fragment. (i) Sequences related to mammalian NF-κB response element: (○) two 17-bp repeats harbouring a decameric κB-related sequence 5'-GGGGATTCCT-3'; (●) a decameric κB-related sequence 5'-GGGAAATTCC-3'; (ii) sequences related to mammalian NF-IL6 response elements: (■) NF-IL6 consensus 5'-TT/GNNGNAAT/G-3'; (□) IL6 RE-BP consensus 5'-CTGGGA-3'. Footprinting experiments have been recently performed [32] with a DNA fragment comprising the four most proximal binding motifs and have confirmed that they are actually protected against DNase I digestion by extracts of fat body from bacteria-challenged larvae (but not from control insects). The possible protection of the more upstream sequences has not yet been tested.

level of several plasma proteins, known as acute phase proteins, many of which act as antiproteinases, opsonins, blood-clotting and wound-healing factors. These proteins are mainly synthesized in the liver and to a lesser extent in extrahepatic cell types which include monocytes, tissue macrophages and fibroblasts.

The coordinate expression of the acute phase protein genes in mammals is at present the focus of intense research. We are similarly interested in the analysis in *Drosophila* of the control of the expression of the antibacterial peptides, which mimics that of acute phase reactants. We have selected the diptericin gene as a model system. *Drosophila* contains one single, intronless, gene encoding an 82-residue diptericin. The gene is rapidly induced in the fat body cells upon bacterial challenge [21]. To dissect the regulation of this gene, we have first transformed flies with a fusion gene in which the reporter β-galactosidase gene is under the control of 2.2 kb upstream sequences of the diptericin gene. We have shown that this fusion gene is inducible by injection of bacteria and respects the tissue-specific expression pattern of the resident gene [30]. To narrow down the functional analysis of potential *cis*-regulatory elements within this 2.2 kb fragment, we have next performed in vitro footprinting experiments on 300 bp of DNA immediately upstream of the TATA box [32]. Four distinct stretches of DNA were found to be protected against DNase I digestion by protein extracts from fat body of bacteria-challenged *Drosophila* adults, but not from control insects (Fig. 2). The protected motifs are: (i) two 17-bp repeats harbouring a consensus motif for binding of NF-κB, a mammalian inducible transactivator involved in the control of expression of immune genes; NF-κB related motifs have been found in all genes encoding inducible antibacterial peptides in insects since the initial report of Faye and co-workers on their presence in the attacin gene promoter of *Hyalophora cecropia* [33]. In mammals, the function of NF-κB is to rapidly induce gene expression upon extracellular stimulations that signal distress and pathogen invasion (reviewed in [34]); in particular, NF-κB controls the expression of acute phase proteins in the mammalian liver and κB motifs can serve as response elements for LPS; (ii) two consensus motifs for binding of NF-IL6; this protein is a member of the C/EBP family of inducible transactivating proteins and has been associated with acute phase regulation [35]. Sequences identical to the protected NF-IL6 consensus motifs of the diptericin promoter are also present in the genes coding for other inducible antibacterial peptides of insects.

We are in the process of investigating the functional relevance of these motifs which are protected in induced tissues. So far we have analysed the role of the 17-bp repeats which harbour a κB-related decameric consensus sequence. We have demonstrated that the replacement of the two 17-bp repeats by random sequences abolishes bacteria inducibility in transgenic fly lines [36]. In association with E. Gateff we have shown that in a tumorous blood cell line [37], the diptericin gene can be induced by addition of lipopolysaccharide (see also [38]). We have transfected these cells with plasmids in which upstream sequences of the diptericin gene were fused to a chloramphenicol acetyl transferase gene and observed a marked induction of the fusion gene by LPS when the 17-bp repeats were present. The replacement of both or either of the 17-bp motifs reduced dramatically LPS inducibility, whereas multiple copies significantly increased the level of transcriptional activation by LPS challenge [36].

In keeping with the data of the footprinting analysis, a specific DNA–protein binding activity could be evidenced when the 17-bp probe was incubated with cytoplasmic and nuclear extracts of induced tumorous blood cells or fat body from bacteria-challenged larvae. No DNA-binding activity was observed in extracts of non-induced cells or fat body of unchallenged larvae nor gut of challenged larvae [36]. The characterization of the transactivating proteins mediating the immune response via the 17-bp repeats is in progress. Our present data lead us to propose that the NF-κB/rel related morphogen dorsal is involved in the activation of the diptericin gene [32].

As already mentioned, during the past few years, the *cis*-acting response elements of several mammalian

acute phase genes have been identified. Sequence similarity among analogous elements has been recognized and several candidate transcription controlling factors for acute phase reactant genes have been identified. These factors were found to be related either to NF-κB or to C/EBP [35]. The observations that consensus binding motifs for members of these two families are present in the immune genes of insects and that they are protected in induced tissues of *Drosophila*, as well as our experimental demonstration that κB-related motifs play a pivotal role in the induction of the diptericin gene in this species, lend strong credibility to the proposal that the insect host defense is homologous to the mammalian acute phase response.

An essential question which we have not yet addressed is the following: how is this rapid non-adaptive response initiated in *Drosophila*. Although, to our knowledge, few structural data are available on immune response receptors of *Drosophila* [39], an increasing amount of information from other arthropod species points to the existence of non-clonally distributed receptors for common constituents of pathogenic microorganisms, such as LPS [40,41], mannans, peptidoglycans [42]. The binding of such conserved microbial constituents by evolutionary primitive receptors (which are conserved in present-day mammals) certainly represents a major recognition event which elicits the immune response in *Drosophila*.

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