Review TRENDS in Parasitology Vol.22 No.6 June 2006

Antimicrobial peptides in the interactions between insects and flagellate parasites

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Innate immunity has a key role in the control of microbial infections in both vertebrates and invertebrates. In insects, including vectors that transmit parasites that cause major human and animal diseases, antimicrobial peptides (AMPs) are important components of innate immunity. AMPs are induced upon parasitic infections and can participate in regulating parasite development in the digestive tract and in the hemolymph. This review presents our current knowledge of a field that is in its infancy: the role of innate immunity in different models of insects infected with flagellate parasites, and in particular the potential role of AMPs in regulating these parasitic infections.

Insect vector–parasite interactions

Insects transmit numerous parasites to humans and animals, and these can cause major diseases, such as malaria, trypanosomiasis, leishmaniasis and filariasis. Within the insect vectors, parasites have specific locations in which to develop: flagellate parasites, such as *Leishmania* spp. and *Trypanosoma cruzi*, develop exclusively in the digestive tract [1,2], African trypanosomes develop in both the digestive tract and salivary glands without entering the hemolymph [3], and certain trypanosomes develop in both the digestive tract and hemocoel before entering the salivary glands [4]. Many non-flagellate parasites also undergo some development within the digestive tract before invading the hemocoel, infecting the salivary glands (*Plasmodium* sp.) [3] or the mouth parts of the vector (filarial worms) [3], and being transmitted to the vertebrate host. During their development within insects, the parasites undergo great morphological changes, and they must also change their surface molecules that enable interactions with specific insect tissues essential for their survival, development and subsequent infectivity to the vertebrate host [3,5].

Many studies have been performed on the physiology, development and ecological interactions between vectors and the parasites they transmit. More recently, the molecular basis of these interactions has become a major field of research. Indeed, a better understanding of the complex biochemical and molecular interactions between insects and parasites could help to develop new strategies to fight the transmission of diseases, including the use of transgenic or paratransgenic (harboring symbionts that express foreign genes) insects [6–8].

The success of vector–parasite interactions depends largely on the immune response of the insect vectors. Insects do not have the antigen–antibody complexes characteristic of the adaptive immunity of vertebrates but have defense mechanisms that rely only on cellular and humoral components of their innate immunity. This system needs to be highly efficient if the insects are to survive in hostile environments. Insects recognize unique pathogen-associated molecular patterns (PAMPs), characteristic of microbial organisms [9], using host molecules called pattern recognition receptors (PRRs) [10]. Two major PRRs in insects are the peptidoglycan recognition proteins (PGRPs) and the Gram-negative bacteria binding proteins (GNBPs) [11]. To date, no such receptor has been identified for parasites.

Once specific PRRs are activated by the appropriate PAMP, signaling cascades are initiated. *Drosophila melanogaster* has been a model of choice for the study of innate humoral responses to bacteria and fungi because of its genetic tool box [12]. In *Drosophila*, challenge with fungi and Gram-positive bacteria activates the Toll pathway, which results in the NF-κB-like transcription factor Dif being translocated to the nucleus and induction of the expression of the gene for the antifungal protein drosomycin. On the other hand, lipopolysaccharide (LPS) present on Gram-negative bacteria is a PAMP that is recognized by the receptors in the immune deficiency (IMD) pathway, which results in the nuclear translocation of Relish (another NF-κB-like transcription factor) and induction of AMPs such as cecropin, drosocin and diptericin [13,14]. These activation processes also trigger various proteolytic cascades that result in melanization and coagulation [11] as well as cellular-mediated mechanisms, including phagocytosis, nodulation and encapsulation by hemocytes [15]. In addition, the humoral response can contribute to the release of reactive intermediates of nitrogen or oxygen, which themselves are lethal to many parasites [16]. This coordinated, multifaceted and integrated approach to protecting the insects from developing pathogens is very efficient, and

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Available online 25 April 2006

 1471-4922/$ - see front matter doi:10.1016/j.pt.2006.04.003

large numbers of bacteria can be removed within minutes of entry into the hemocoel [17].

A major component of this successful immune response is the rapid expression of AMPs, which have been described in plants [18], invertebrates [19] and vertebrates [20]. The ubiquity and strong conservation of AMPs indicates a crucial role for these molecules in innate immunity. Some AMPs, such as the defensins, are well-represented in all of these phyla, whereas other AMPs, such as the cecropins, are restricted to very few taxa [18–20]. Many research papers have focused on the role that AMPs play in insect innate immunity. For example, upon pathogen infection, *Drosophila* can discriminate between fungal and bacterial infections and synthesizes specific lethal AMPs. The role of AMPs in regulating parasite development is best studied in mosquitoes [11], because of their importance in transmitting parasites that cause malaria and filariasis. Less well studied, but equally important, are vectors that transmit kinetoplastid parasites, which also cause significant disease in humans and animals: the *Trypanosoma brucei* complex causes sleeping sickness in Africa, *Trypanosoma cruzi* causes Chagas disease in Latin America and *Leishmania* sp. is responsible for cutaneous and visceral diseases worldwide. The role of AMPs in the innate immunity of vectors to these different flagellate parasites is limited by the relatively few studies done on the vectors of these parasites. The current knowledge is summarized here.

Insect–flagellate interactions

The immune response of insects to flagellates was long considered to be mediated mainly by lectins [3]. Following the discovery of AMPs as part of innate immunity, studies began to investigate their influence on flagellate parasite development. Flagellate parasites are particularly interesting models because most of them do not invade the hemolymph, as do parasites such as *Plasmodium* sp. or filarial nematodes. For this reason, the induction of AMP synthesis cannot be attributed to migration of the parasite to the hemolymph and the associated tissue damage. The immune response of insect vectors to flagellates has been studied in various models: *Crithidia* parasitizing *Drosophila, Trypanosoma* sp. parasitizing *Glossina* sp., *Leishmania* parasitizing *Phlebotomus* sp., and *Trypanosoma cruzi* parasitizing *Rhodnius prolixus.*

Fecal transmission

In *Drosophila* sp. and *Rhodnius sp.*, parasites develop exclusively in the digestive tract and are transmitted through the feces.

Drosophila and *Crithidia*: Few parasites develop in *Drosophila*, but *Crithidia* sp. does and is transmitted directly from insect to insect via feces. The full development of the parasite within the digestive tract takes only a few days (Table 1, Figure 1a) (A.Y. Ismaeel, PhD thesis, University of Liverpool, UK, 1994). In *Drosophila,* eight different AMP families have been identified after infections with fungi or bacteria and the activation of the Toll and IMD pathways. Each AMP has distinct antimicrobial properties and a different spectrum of organisms on which it can act [13]. Upon parasitic infections with *C. fasciculata* or *C. bombi*, these AMPs are also induced [21] (Figure 2).

Kissing bug (*Triatoma* sp. and *Rhodnius* sp.) and *Trypanosoma cruzi*: Parasites are ingested with the blood meal and develop into infective stages that migrate to the rectum, forming infective stages in the rectum. As the insect feeds, it engorges and defecates: parasites in the feces fall on the skin of a potential host and can enter via skin abrasions or via mucous membranes [2] (Table 1, Figure 1b). Upon parasite infection, the only AMP isolated from these vectors is a defensin produced in the fat body and midgut. This defensin is induced after local and systemic infection (Figure 2) [22].

Salivary transmission

In two other insects, *Glossina* sp. and *Phlebotomus* sp., parasites are acquired via a blood-sucking (hematophagous) bite but are not transmitted via the feces. Instead, they migrate up the digestive tract and are transmitted

Table 1. Parasite development in insects

Models	Insect forms[a]	Vertebrate forms[a]	Transmission mode	Time of development in insect	Refs
Drosophila and *Crithidia*	Choanomastigotes in the digestive tract	-	Direct by the feces	5–7 days	[b]
Glossina and *Trypanosoma brucei*	Epimastigotes and procyclic trypomastigotes in gut and metacyclic trypomastigotes in salivary glands	Trypomastigotes in blood and cerebrospinal fluid	Hematophagous bite; salivary transmission	20 days	[3,23]
Phlebotomus or *Lutzomia* and *Leishmania* spp.	Promastigotes in midgut then in anterior midgut	Amastigotes in reticulo-endothelial system	Hematophagous bite; salivary transmission	10 days	[1]
Triatoma or Rhodnius and *Trypanosoma cruzi*	Epimastigotes in intestine and trypomastigotes in rectum	Trypomastigotes in blood and tissues and amastigotes in striated and smooth muscles	Hematophagous bite and excretion in the feces of the infective forms; fecal transmission	1–2 weeks	[2]

[a]The choanomastigote stage has a 'pear shape' with an anterior flagellum. The epimastigote stage is a long form with a juxta-nuclear insertion of the flagellum. The trypomastigote stage has a flagellum inserted at the posterior end and making an undulating membrane. The promastigote has an anterior insertion of the flagellum, and the amastigote has a round shape, no flagellum and is an intracellular parasite.

[b]Ismaeel, A.Y.(1994) Studies on host–parasite relationships of trypanosomatid flagellates in Drosophila, and Leishmania in Phlebotomines sandflies. PhD thesis, University of Liverpool, UK).

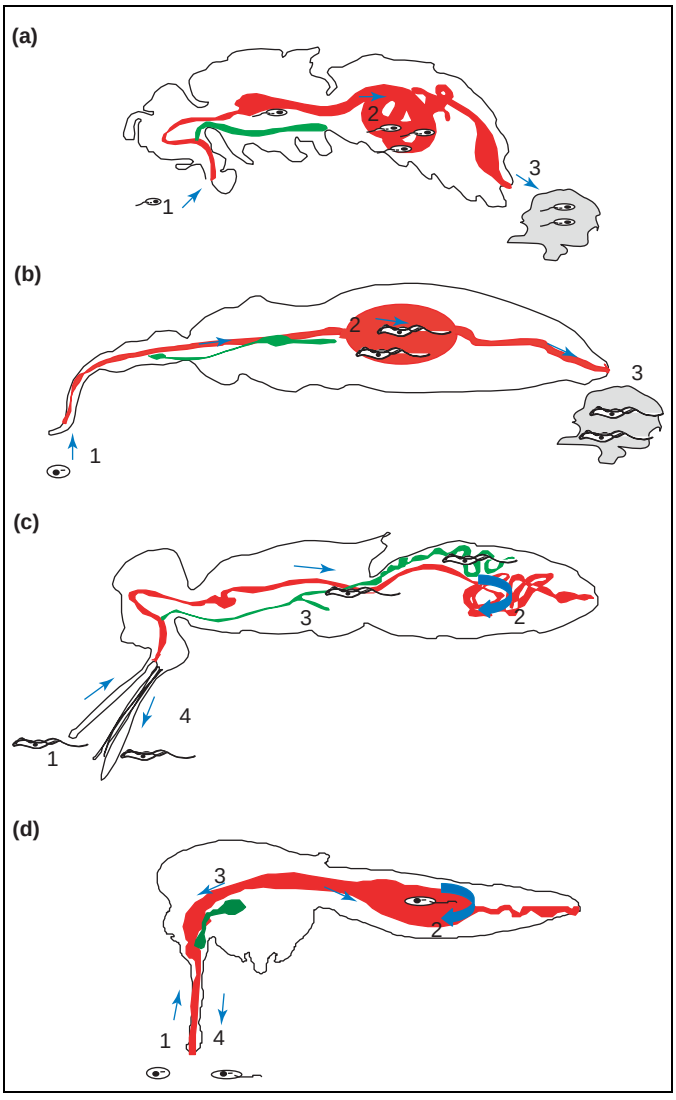

Figure 1. Migration of flagellates in their insect vector: **(a)** Model of *Drosophila* and *Crithidia*; **(b)** model of kissing bug (*Rhodnius*) and *Trypanosoma cruzi*. Parasites, after migration through the digestive tract, are excreted with the feces (a,b). **(c)** Model of *Glossina* and *Trypanosoma brucei*; **(d)** model of *Phlebotomus* and *Leishmania*. Parasites migrate through the digestive tract. However, for *Glossina* (c), the parasites go around the peritrophic membrane and then accumulate in the salivary glands. For *Phlebotomus* (d), the parasites are stored in the anterior gut, without entering the salivary glands. Red: indicates the digestive tract; green indicates the salivary glands. Numbers indicate the different steps of parasite migration.

during blood feeding without ever entering the hemocoel (for *Phlebotomus* infected with *Leishmania*). For *Glossina*, most trypanosome species develop first as procyclic trypomastigotes (non-infectious forms with surface procyclin proteins) in the midgut and then as metacyclic trypomastigotes (the infectious forms with a variant surface glycoprotein coat) in the salivary glands. Both *Trypanosoma* and *Leishmania* are transmitted to the vertebrate host by a hematophagous bite.

Glossina and *Trypanosoma:* The tsetse fly, *Glossina*, is a vector of *T. brucei*, which causes sleeping sickness in humans and nagana in cattle in Africa [23]. Trypanosomes multiply first in the digestive tract of the tsetse fly and undergo a complex migration around the peritrophic matrix (a chitin-containing structure that protects the midgut wall) before invading the salivary glands about 20 days after ingestion (Table 1, Figure 1c). The presence of molecules with antibacterial activity in the hemolymph of the tsetse fly was suspected for a long time [24,25]. Recently, several AMPs (defensin, cecropin, attacin and diptericin) [27,28] (Figure 2) were characterized in *G. morsitans* infected with bacteria or *T. brucei*. They are induced only in the first week after infection, with diptericin constitutively expressed and upregulated upon the infective blood meal [26].

Phlebotomus and *Leishmania:* In the sandfly vector (*Phlebotomus* sp. in the Old World and *Lutzomia* sp. in the New World), *Leishmania* parasites multiply intensively in the digestive tract before they accumulate behind the stomodeal valve in the anterior midgut for the transmission to the vertebrate host [1] (Table 1, Figure 1d). A single AMP, a defensin, has been identified in *P. duboscqi* infected with bacteria or with *L. major*. This defensin is strongly induced 4 days after the infective blood meal [28] (Figure 2). Strikingly, defensin induction was greatly reduced after infection with two *L. major* mutants, *lpg1*$^{-}$ and *lpg2*$^{-}$. The parasite mutant *lpg1*$^{-}$ lacks a putative galactosylfuranose responsible for the formation of the lipophosphoglycan (LPG) core [29], whereas *lpg2*$^{-}$ is defective in the synthesis of all phosphoglycans [30]. Surface antigens of *Leishmania* parasites might impair the ability of the insect vector to recognize the parasite as non-self.

Structure and biological properties of AMPs

Biochemical approaches (reverse-phase high-performance liquid chromatography and mass spectrometry analysis) associated with *in vitro* antimicrobial assays have been used to study induction of AMPs in immune-challenged insects [31]. Interestingly, after infection *per os* with bacteria or parasites, AMPs were detected locally in the gut, the main site of flagellate infections, but also systemically in the hemolymph, where no parasite is found [21,22,26–28]. Whereas AMP concentrations reach their peak around 24 hours after bacterial infections, the kinetics and pattern of AMP induction following parasite ingestion varies according to the stage of parasite development, suggesting a possible role for surface molecule variation of the parasite in this induction.

In dipteran insects, AMPs are synthesized principally by the fat body and released into the hemolymph, but they can also be expressed by the hemocytes and various epithelia, particularly the anterior part of the gut [32,33]. Hamilton *et al.* [34] showed that defensins of the blood-sucking fly *Stomoxys calcitrans* are secreted into the gut. The AMP is therefore in contact with the blood meal, bacteria or parasites. Midgut defensins are bound in a stable complex to a serine protease, from which they are released when secreted into the gut lumen [34]. In *S. calcitrans*, the concentration of AMP was estimated to be 530 pg per gut [35]. In mammals, the local concentration of AMPs in the gut (Paneth cells) can reach the mg/ml level [36]. As all the flagellate parasites develop in the digestive tract, a direct effect of secreted AMPs on flagellates is likely to occur.

Although various insect AMPs are produced by all of these vectors, and many are likely to be species-specific,

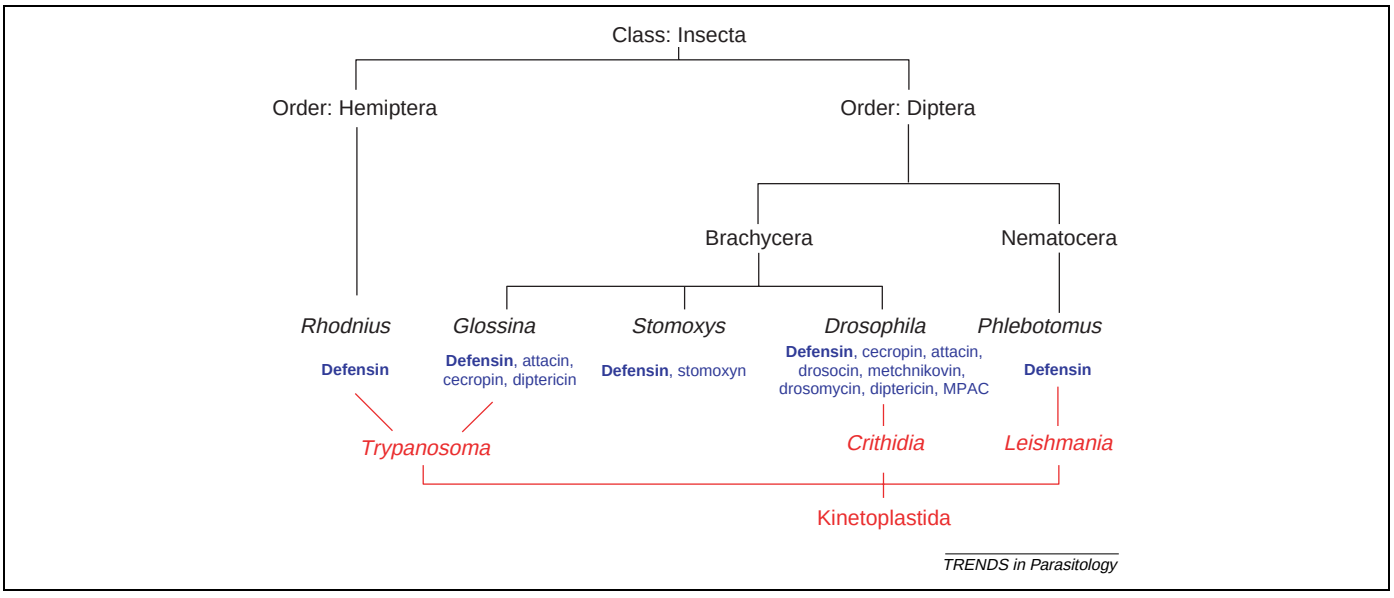

Figure 2. AMPs identified in the different insects upon pathogen infections (bacterial or parasitic). Parasites are in red, AMPs in blue and insects in black; MPAC, mature prodomain of attacin C. The relationships between the insects and the parasites discussed is shown by phylogenetic trees.

the most common AMP in all these different models is defensin (Figure 2). Defensins of dipteran insects are cationic peptides of 33–46 amino acids. The *Glossina* defensin is the smallest, with 33 amino acids (Figure 3), and an N-terminally extended defensin has been reported in the gut of the *S. calcitrans* [32]. Most insect defensins are characterized by six invariant cysteine residues arranged in three intra-molecular disulfide bridges. This leads to a compact 3D structure consisting of a N-terminal loop, an α-helical domain linked to two twisting antiparallel β strands by two disulfide bridges [19]. Defensins are active mainly against Gram-positive bacteria but can also be active against Gram-negative bacteria, fungi [19] or parasites [28,37]. Most of the antibacterial defensins kill bacteria in less than a minute, often at minimal inhibitory concentrations below 1 μM. Different isoforms of defensins, which show temporal or spatial differences in expression pattern, are also found in vectors; defensin C from *Aedes aegypti* is present in the midgut of naive insects, and defensin isoforms A and B are induced in the fat body of immunized insects [38].

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Order Diptera
          Suborder Nematocera
Aedes aegypti A        ATCDLLS----GFGVGDSACAAHCIARGNRGGYCNSKKVCVCRN
Aedes aegypti B        ATCDLLS----GFGVGDSACAAHCIARGNRGGYCNSQKVCVCRN
Aedes aegypti C        ATCDLLS----GFGVGDSACAAHCIARRNRGGYCNAKKVCVCRN
Aedes albopictus D     ATCDLLS----GFGVGDSACAAHCIARRNRGGYCNAKKVCVCPI
Anopheles gambiae      ATCDLAS----GFGVGSSLCAAHCIARRYRGGYCNSKAVCVCRN
Phlebotomus duboscqi   ATCDLLS----AFGVGHAACAAHCIGHGYRGGYCNSKAVCTCRR

          Suborder Brachycera
Stomoxys calcitrans    ATCDLLS----MWNVNHSACAAHCLLLGKSGGRCNDDAVCVCRK
Stomoxys calcitrans    ITCDLLS----LWKVGHAACAAHCLVLGNVGGYCT---------
Stomoxys calcitrans    ITCDLLS----LWKVGHAACAAHCLVLGDVGGYCTKEGLCVCKE
Glossina morsitans     VTCN----------IGEWVCVAHCNSKSKKSGYC-SRGVCYCTN

Order Hemiptera
Rhodnius prolixus A    ATCDLFSFRSKWVTPNHAACAAHCLLRGNRGGRC-KGTICHCRK
Rhodnius prolixus B    ATCDLLSFSSKWVTPNHAGCAAHCLLRGNRGGHC-KGTICHCRK
Rhodnius prolixus C    ATCDLLSLTSKWFTPNHAGCAAHCIFLGNRGGRC-VGTVCHCRK
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Figure 3. Amino acid sequences of some selected defensins from insect vectors of parasites (except *Stomoxys calcitrans*). Identical or conserved amino acids are in bold. Conserved amino acids are shaded, cysteine residues are in bold, and the dotted lines show gaps. References are as follows for defensin sequences: *G. morsitans* [27]; *P. duboscqi* [28]; *R. prolixus* [22]; *S. calcitrans* [32]; *Aedes aegypti* A,B,C [57]; *Aedes albopictus* [58]; *A. gambiae* [59]. The SwissProt accession number for the *S. calcitrans* defensin Smd-1 is P82380.

AMPs during infection of insects with flagellates

In these different models, some aspects of innate immunity are constant. It is now clear that ingested parasites induce a local immune response (in the gut tissue) that can be followed by a systemic (hemolymph) immune response [21,22,26–28,32]. On the other hand, a systemic infection induced by a septic injury through the insect cuticle triggers a systemic as well as a local immune response in the gut [22,32]. The organization and physiological significance of these phenomena are not clearly understood. A concrete understanding of how AMPs are induced in tissues that have no contact with pathogens, the multifunctional role of AMPs, and the signaling molecules involved in inducing the systemic responses are imperative if we are to understand fully how insects, and other organisms, survive in the presence of pathogens.

Role in the control of parasite development

Parasites transmitted by insect vectors occupy a specific niche in the insect environment. In order to be transmitted to the vertebrate host, they must establish a relationship in which neither the insect nor the parasite dies. Depending on their life cycle, parasites must face the insect immune response first in the gut and then, if they cross an epithelial barrier, in the hemolymph. Insect AMPs are most often described as molecules with antibacterial and antifungal activities [19] and as such, they can directly protect the insect from bacterial and fungal infections, but the same AMPs might also protect parasites from pathogenic bacteria. Indeed, it has been shown in different insects that a co-infection of parasites with pathogenic bacteria has a lethal effect on the parasites [39–41]. However, insect gut microbiota that have an obligate and mutualistic relationship with the insect they inhabit do not trigger AMP synthesis [41].

Some AMPs also have direct antiparasitic activity on flagellate parasites. A lethal effect has been shown in several heterologous systems: *Hyalophora* cecropin has

a lethal effect on *T. cruzi* [42], spider gomesin and frog temporins on *Leishmania* [43,44] and *Phormia* diptericin on *Trypanosoma* spp. [26]. However, it should be noted that *in vitro* studies used to determine lethal concentrations of heterologous peptides sometimes use concentrations far in excess of what the parasites will encounter under normal physiological conditions. Parasites in their normal vector might never be exposed to such high concentrations, or they could be tolerant of the AMP to which they normally are exposed but susceptible to similar peptides from other insects.

An effect of AMPs in natural associations of parasites and vectors was observed more recently, suggesting a possible role of AMPs in vector competence. In the sandfly, *P. duboscqi,* a defensin was found to be active specifically on the promastigote forms (insect forms) of *L. major* [28]. A recombinant *Glossina* attacin was also shown to have trypanolytic activity against the blood stages and the insect forms of *T. brucei, in vitro* and *in vivo* [45].

AMPs might contribute to the specificity of the parasite–vector interaction. Preliminary studies in which *P. duboscqi* defensin was tested on different *Leishmania* species (*L. major, L. donovani* and *L. infantum*) revealed that the antiparasitic activity of the *P. duboscqi* defensin was specific to the parasite normally transmitted by that insect (R. Brun and N. Boulanger, unpublished). These data suggest that the parasites are recognized by the insect immune system and that AMPs might be involved in determining the specificity of parasite–vector associations by regulating parasite numbers (good vector competence) or by directly killing parasites (poor vector competence). As an example of poor vector competence, the analysis of the gut tissue of *S. calcitrans* is relevant. In this insect, stomoxyn (an α-helical peptide of 42 amino acids) is expressed constitutively in the anterior part of the gut. This AMP was found to kill the trypomastigote forms (vertebrate forms) of *Trypanosoma brucei rhodesiense* [35]. Interestingly, *S. calcitrans* is found in the same habitats as the tsetse fly vector of *Trypanosoma* spp. and the two dipterans share a very similar gut physiology and feed on the same hosts for a blood meal, but only the tsetse fly, which does not have a stomoxyn-like peptide in its genome, is a vector of sleeping sickness. Stomoxyn might contribute to the refractoriness of *S. calcitrans* to trypanosomes. This could be tested by knocking out stomoxyn using RNA interference (RNAi) technologies and exposing *S. calcitrans* to a parasite-infected host.

Role in sterilization of the ingested meal

The broad distribution of AMPs suggests that they also must have an important role in the physiology of the insect. The fact that the gut is a site of AMP synthesis is particularly interesting and deserves further study. Specifically, the anterior part of the gut was found to be the site of AMP synthesis for a range of insects [22,32,33,46]. This part of the gut is, in fact, involved in the sterilization and dehydration of the blood meal before further digestion [47]. For some hematophagous insects, the blood meal is the single source of nutrients and is necessary for the maturation of their eggs [48]. Therefore, the protection of the blood meal from possible microbial infections is essential for insect survival. AMPs synthesized in gut cells are further processed and cleaved in the gut lumen [34], where a direct effect on ingested microorganisms can occur. This production and release of AMPs into the gastrointestinal tract could be a general response of the insects to reduce the pathogenic bacteria ingested with the blood and reduce potential microbial infections, which would otherwise be lethal for the insect.

Roles other than as antimicrobial molecules

AMPs could have other biological functions in insects, as they do in mammals. In vertebrates, AMPs induce proteoglycan expression during wound repair [49], can have cytotoxic and apoptotic properties on mammal cells *in vitro* (cathelicidin [50]) and can chemoattract neutrophils and macrophages (defensin [51]). Such a multifunctional role of these molecules is even more important in small organisms. AMPs and nitric oxide also serve as signaling molecules between the anterior part of the gut and the fat body [52,53]. This communication between these two important immune organs might maintain the control of infections and enable insect survival in hostile environments. Bartholomay *et al.* [54] propose that mosquito defensins might have a role as stress proteins when the level of pathogens becomes too high to be controlled by cellular responses. Insect defensins, therefore, might have chemotactic properties to bring hemocytes to sites of infection, as do their mammalian counterparts [51].

Studies using homologous models that mimic the natural host–pathogen system are now necessary to evaluate the significance of AMPs in insects [54]. Using available technologies, such as RNAi and generation of parasite mutants, should help to investigate additional roles of AMPs in insects infected with flagellates. In mosquitoes, silencing the expression of defensin using RNAi indicates that this AMP by itself is not directly involved in controlling *Plasmodium* infections, although the silencing does not make the mosquito more sensitive to bacterial infections [55]. These data indicate that defensin is not required to eliminate bacteria from the hemocoel, but they do not indicate a concrete role or rationale for the simultaneous expression of several AMPs with different modes of action, to eliminate a single pathogen infection. Parasite mutants, already available for *Leishmania* [29,30] and African trypanosomes [5], should also help to investigate the exact role of parasite surface antigens in the induction and the regulation of AMPs in flagellate parasite infections.

Future perspectives

The discovery of AMPs has increased our understanding of basic insect immunity and also their role – in conjunction with lectins, proteolytic cascades, digestive enzymes and the peritrophic matrix – in regulating and controlling parasite development. There is ongoing research in several laboratories into the direct and indirect interactions between parasites and AMPs, especially the well-studied AMP defensin. This molecule, found so ubiquitously in the innate immune systems of

different groups (plants, invertebrates and vertebrates), probably has a fundamental role in the biology of organisms. The fact that its absence (after RNAi) has no effect on bacterial and *Plasmodium* infection in mosquitoes [55] suggests that AMPs such as defensin might have other roles in insect physiology. The phenotype of such AMP-deficient insects should be studied more thoroughly.

A major model for the study of insect AMPs is *Drosophila,* but this species harbors very few parasites. However, the available *Drosophila* AMP mutants could be used to unravel mechanisms of innate immunity towards parasites isolated originally from this insect (A.Y. Ismaeel, PhD thesis, University of Liverpool, UK, 1994). Indeed, so far, *Crithidia* species isolated from mosquito (*C. fasciculata*) and bumble bee (*C. bombi*) infecting *Drosophila* AMP mutants did not give conclusive results. Because the genomes of most insects that transmit flagellate parasites have not been sequenced, we must use other available tools to analyze more precisely the role of AMPs in regulating parasitic infections. Using available mutants of *Leishmania* and *Trypanosoma* will enable us to determine how flagellates parasites are recognized by the insect immune system and what factors induce AMPs. Studies on AMP induction during co-infections of flagellate parasites with pathogenic and non-pathogenic bacteria should also help to establish the real target of AMPs during these co-infections. Understanding how parasites are recognized as non-self, and how closely related parasites (such as *T. cruzi* and *T. rangeli*) have developed different life cycle and transmission strategies (fecal versus salivary) in the same vector species in the face of common immune responses is essential for our knowledge of vector–parasite interactions and vectorial competence. Finally, AMPs might regulate parasite development directly. In sandflies, defensin is expressed in the greatest amounts 4 days after the infective blood meal, which corresponds to a time of intensive parasite multiplication [28]. The AMP might be secreted to limit parasite population growth below lethal levels, or the parasite might use the presence of the AMP to induce its own multiplication.

AMPs could have a role in the development of new strategies to control arthropod-borne diseases, such as these caused by flagellate parasites. Engineering AMPs in symbionts of tsetse flies or reduvid bugs affects parasite survival [56]. Several AMPs have marked antiparasitic activity and are therefore candidates for use in the development of transgenic insects. Stomoxyn, with its trypanolytic activity on *T. b. rhodesiense*, is an interesting candidate for engineering in the tsetse fly to investigate vector competence. This technology is now feasible and is a promising tool in the control of parasite infected insects.

Acknowledgements

We thank Reto Brun, Laurence Sabatier, Mike Lehane, Petr Volf, Raul Ursic and Stephen Beverley for their contributions. This research was supported by CNRS, Atheris and Entomed, NIH-AI44966, NSERC-261940 and CIHR-69558 grants to C.L.

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