ISSN 0022-0930, Journal of Evolutionary Biochemistry and Physiology, 2013, Vol. 49, No. 1, pp. 1—9. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © R.A. Ilyasov, L.R. Gaifullina, E.S. Saltykova, A.V. Poskryakov, A.G. Nikolaenko, 2012, published in Zhurnal Evolyutsionnoi Biokhimii i Fiziologii, 2012, Vol. 48, No. 5, pp. 425—432.

REVIEWS

Defensins in the Honeybee Antiinfectious Protection

R. A. Ilyasov, L. R. Gaifullina, E. S. Saltykova, A. V. Poskryakov, and A. G. Nikolaenko

Institute of Biochemistry and Genetics, Ufa Research Center, Russian Academy of Sciences, Ufa, Bashkortostan, Russia
E-mail: apismell@hotmail.com

Received 10 October, 2011

Abstract—Specific conditions of the honeybee life honeybee life require the presence of effective mechanisms of antiinfectious protection whose one of the most important components are defensins—the family of antimicrobial peptides. In the honeybee, defensins are present in the form of two different peptides—defensin 1 and 2 that are similar between each other only by 55.8%. Defensin 1 synthesized in salivary glands plays an important role in social immunity, whereas defensin 2 synthesized by cells of fat body and lymph is an important factor in the system of the honeybee individual immunity. Defensins are inducible, are controlled by interaction of Toll and Imd signal pathways and have a large specter of antimicrobial action.

DOI: 10.1134/S0022093013010015

Key words: honeybee, *Apis mellifera,* antimicrobial peptides defensins, evolution, immunity.

INTRODUCTION

The family of honeybee *Apis mellifera mellifera* L. is represented by more than 10 thousand individuals in the limited space of the hive. The constant temperature of 37°C inside the hive and frequent contacts of individuals created the ideal medium for fast spreading of infectious diseases [1]. These life conditions promoted the appearance of different mechanisms of antiinfectious protection. Apart from the individual protection systems, the collective mechanisms of resistance were developed in social insects in the process of evolution to compensate the increased risk of infection by the pathogenic microorganisms in the common life style. These protective mechanisms were called "social", "public" or "collective immunity" [2]. The components of the honeybee humoral antiinfectious protection are expressed in hemolymph [3], fat body [4], and cuticle [5], in middle intestine [6] and in salivary glands [7].

The antimicrobial peptides (AMP) are important components of the honeybee immune system [3]. This is one of the evolutionary ancient and important mechanisms of invertebrates. AMP can be activated for the short time interval and be delivered to the site of infection [8]. By the strength of their action, AMP are compared with antibiotics and can be used for the elaboration of the medications that have antifungal and antibacterial properties [9]. The use of the antifungal preparations and antibiotics at treatment of honeybee diseases leads to suppression of their own immunity, to the appearance of pathogens stable to them, and to pollution of products of apiculture. Thus, T. Miyagi with coauthors (2000) [10] showed development of resistance of the pathogenic for honeybees bacteria of the American foulbrood *Paenibacillus larvae larvae* to the antibiotic oxytetracycline. Solution of this problem is an enhancement of the honeybee immunity by an increase in the AMP expression level AMP in the honeybees themselves [11]. Unfortunately, data on study of defensins that have the greatest polymorphism

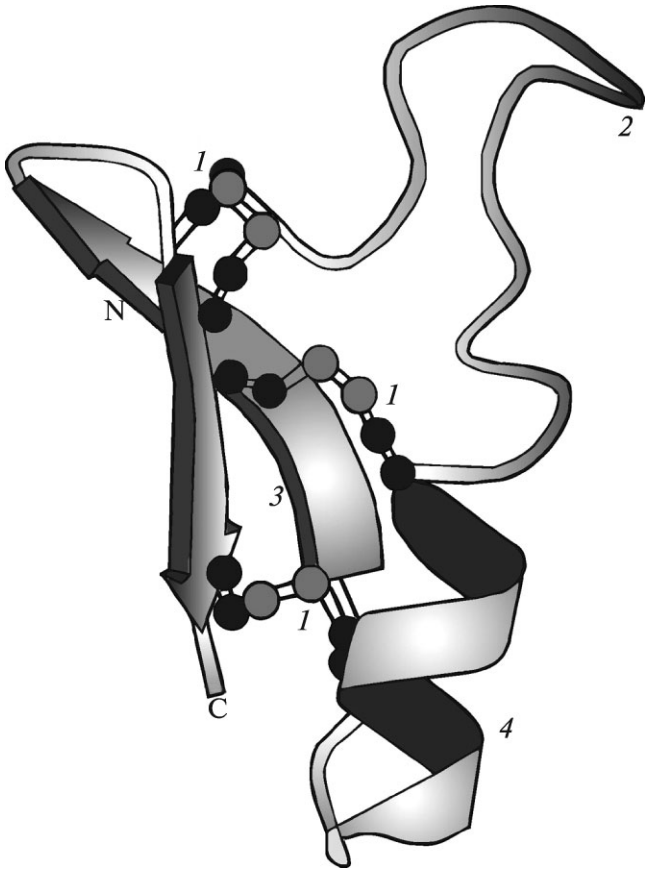

Fig. 1. The spatial structure of insect antimicrobial peptide defensin (from [24]). (*1*) Three cysteine disulphide bridges; (*2*) loop; (*3*) two antiparallel β-layers; (*4*) α-helix.

and specter of the effect out of all honeybee AMP are fragmentary and do not provide the integral picture about their role in the life activity of public insects and, particularly, in the antiinfectious protection of *Apis mellifera* L.

STRUCTURE AND TISSUE-SPECIFIC EXPRESSION OF DEFENSINS

The defensins represent family of cysteine-reach AMP. The defensins of honeybee are homologues of phormicynes of the fly *Phormia terranovae* and differ from other peptides by peculiar structure and also by selective activity with respect to gram-positive bacteria and some mycelial fungi [12, 13].

At present, four AMP species are detected in honeybee: apidaecin [14], abaecin [15], hymenoptaecin [1], and defensins. The defensins are represented by two peptides—defensin 1 and defensin 2 with molecular mass 5.5 and 4.8 kDa, responsively; they are encoded by genes *defensin 1* and *defensin 2*. Defensin 1 has 3 isoforms—defensin of hemolymph and 2 isoforms detected in royal jelly and called royalysine—Ro-F [16] and Ro-K [17] with molecular mass 5525.1 and 5515.5 kDa, respectively. The royalysine differs from the honeybee hemolymph defensin by two amino acid replacements [17]. P. Kwakman with coauthors (2010) [18] showed the antimicrobial properties of honey, which in the 10% dilution kills *Bacillus subtilis*, the resistant to methycillin *Staphylococcus aureus*, the β-lactamase-producing *Escherichia coli*, the cyclophloxacin-resistant *Pseudomonas aeruginosa*, and the vankomycin-resistant *Enterococcus faecium*. It has been shown that antimicrobial properties of honey are provided by the presence of hydrogen peroxidase, methylglyoxal, and the antimicrobial peptide defensin-1.

The gene of defensin 1 (*defensin 1*) is composed of 2012 n.p. (the number in the gene-bank is AY496432) and contains two introns. The first intron with size of 571 n.p. is located between 773 and 1345 nucleotides, while the second—with size 278 n.p. is located between 1525 and 1804 nucleotides. With exception of introns, the gene sequence is completely identical to cDNA of royalysine Ro-K. The sequestration of cDNA showed similarity of royalysine to the gene of defensin 1, except for one site in the 377 nucleotide, where there occurred one-nucleotide replacement (C-T) [19]. The gene of defensin 2 (*defensin 2*) is composed of 1950 n.p. (the number in the gene-bank is AY588474) and contains one intron with the size of 335 n.p. located between the 947 and 1283 nucleotides. The high level of similarity is observed at the DNA level of the central module of defensins. On the other hand, the genes have the low level of similarity over the TATA box and below the TATA box until the site of processing of mature peptide, as well as in the 3'-noncoding region.

Two genes *defensin 1* and *defensin 2* differ significantly by the length, intron-exon structure, and sequence of their pre-pro-regions. Specifically, the second intron of *defensin 1* out of two, which is not represented in *defensin 2*, is the first intron found in the coding part of the defensin gene in arthropods. The short aminated C-terminal elongations are revealed only in the hymenopteran de-

fensins [20, 21]. These elongations from 11 amino acids seem possibly acquiring the defensin molecule form of alpha-helix that stabilizes amino C-ends [14]. The exact role of these elongations in the peptide function is not yet finally elucidated. Possibly, these elongations are a result of exon shuffling [22]. The atypical exon appears as a result of recombination of two exons with the elongation at the C-terminus. Similar processes of the appearance of C-terminal elongations also occurred in the process of evolution in two bumblebee species.

The insect defensins with length of 36 and 46 amino acids, except for defensins of honeybee and bumblebee with length of 51 amino acids, contain three cysteine pairs bound with two disulphide bridges [23, 24] (Fig. 1). Initially, two isoforms of the honeybee defensins were revealed: royalysine from the royal jelly and defensin from hemolymph. The defensin from hemolymph and its precursor propeptide were characterized by cDNA synthesized from RNA prepared from the tissues of honeybee abdomen [19]. The defensin from the royal jelly royalysine was described at the level of amino acids [16]. Further the presence of royalysine was revealed as well as in the hemolymph of honeybee [19]. Both defensin isoforms of honeybee were encoded by one gene and have length of 51 amino acids, but differed by one–two amino acid replacements. J. Klaudiny with coauthors (2005) [17] described two forms of defensins, encoded by different genes, which were called defensin 1 and defensin 2. It turned out that royalysine was a posttranslational variant of defensin 1. Besides, there was detected a new isoform of royalysine that was shorter by 10 Da than the previously known one. Both defensin genes were expressed in the honeybee thorax. However, in the honeybee hypopharyngeal, mandibular, and thoracic glands, only defensin 1 mRNA was revealed, which indicates its tissue-specific expression. Defensins usually are expressed later than other AMP, but their activity is preserved for more than two weeks after the honeybee infection [14].

Defensin 2 has pre-propeptide longer than defensin 1. The mature peptide consists of 43 amino acids and does not contain the C-terminal elongations detected in defensin 1 and other hymenoptera defensins. Its molecular mass is 4808.6 Da. The mature peptide defensin 2 showed only 55.8% of similarity with defensin 1.

M. Yoshiyama and K. Kimura (2010) showed in their work a very high level of cDNA similarity of defensin (AcjDef2) of *Apis cerana japonica* with defensin of *Apis mellifera* (AmDef) (93.0%), which indicates close phylogenetic relationship of these honeybee species [25]. We studied variability of the gene fragment of defensin 1 in populations of honeybee *A. m. mellifera* in Ural [26]. The results of these studies revealed the presence of two alleles of this locus. The allele B of the gene fragment of defensin was exposed with frequency of 0.14–00.25, while the allele A—with frequency 0f 0.75–0.86. Earlier the frequency of the fragment alleles of gene defensin was distributed in the Ural honeybee populations more regularly: the allele B was exposed with frequency 0.25–0.45, while the allele A—0.55–0.75 [26]. These changes in the frequencies of defensin alleles can be due to the occurring microevolutionary processes.

Numerous variants of defensin isoforms can be due to the posttranslation modification under effect of gene in other loci, but not to the one-locus mutation [27]. Possibly, one of causes of the appearance of polyvariability of the defensin antimicrobial activity in honeybees in the process of evolution is variability of processing of the defensin precursors, which leada to the appearance of isoforms.

On the whole, defensins in insects are encoded by the different number of genes: in the fly *Drosophila melanogaster* by one gene [28], in the fly *Rhodnius prolixus*, the carrier of Chagas disease, by three genes [29]. In arthropods, the autonomous modules represented in the exon of mature defensins are developed as a result of some shuffling and fragments integrated with the main sequence [28]. This is proved by comparing defensins of other species at reconstruction of phylogenesis, where, unlike the mosquito defensins, the honeybee defensin genes were paraphiletic (at a high level of significance 84%). Defensin 1 of honeybees formed a group with defensins of two other hymenoptera species—the bumblebees *Bombus pascuorum* and *Bombus ignitus*, whereas defensin 2 was grouped with defensins of beetles *Oryctes rhinoceros* and *Allomyrrhina dichotoma* (Fig. 2) [17]. The outside group served the defensin gene of the mollusc *Mytilus galloprovinciales*. Thus, the defensin genes differ by the number of introns in the gene part encoding the pre-proregions. Differ-

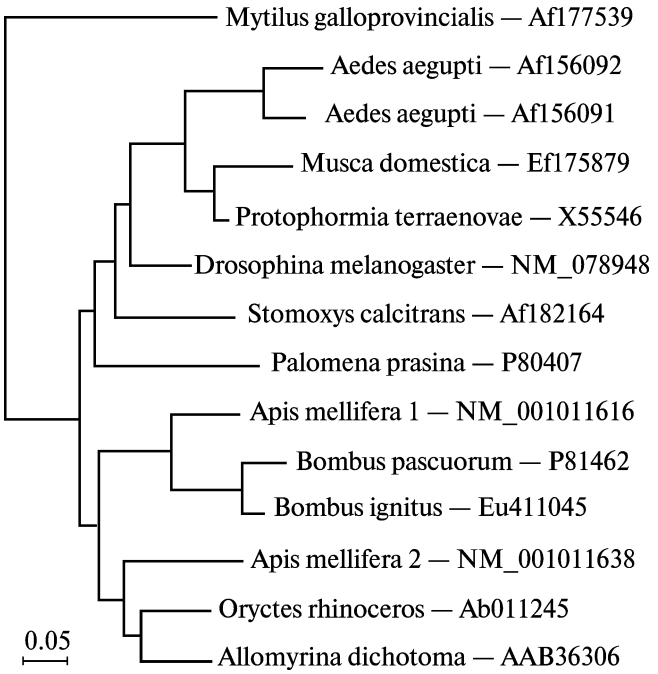

Fig. 2. The phylogenetic scheme constructed on the basis of straightened amino acid sequences of peptide defensin of different vertebrate species (from [17]). The horizontal bar with the digit 0.05 shows scaling of the genetic divergence between species in the scheme.

ences in the exon–intron structure as well as in the sequence of the mature peptide show that, in spite of the common origin, they had the long-term independent evolution.

SPECTER OF DEFENSIN ACTIVITIES

Bacteria. The honeybee defensins have a sufficiently wide specter of antibacterial activity. It is known about cytotoxic activity of defensins against gram-positive bacteria [19, 30] and some species of gram-negative bacteria [31]. One of factors of social immunity is secretion of royalysine with royal jelly for feeding and protection of larvae from bacteria. It has been shown that royalysine is mainly effective against the gram-negative and particularly against the gram-positive bacteria [17]. Particularly, there is known the royalysine activity against the American foulbrood *Paenibacillus larvae larvae* [11, 25, 32].

The Lactobacillus bacteria non-pathogenic for honeybee also stimulate an increase of the expression level of abecin and defensins, which promotes a possibility of their use as probiotics for enhancement of immunity in honeybee [31]. H. Yoon and coauthors [33] showed induction of the defensin gene expression in the fat body of working individuals of other representatives of the Apidae family—three species of bumblebee *Bombus terrestris, B. ardens ardens*, and *B. hypocrite sapporoensis*—by injection of lipopolysaccharides modulating effect of the bacteria infection. It has been similarly shown that chitozans by simulating the microorganism invasion also stimulate the defensin gene expression in honeybee [34, 35].

Fungi. The honeybee defensins have cytotoxic activity against the mycelian fungi and yeast—the fungi of calcified brood *Ascosphaera apis,* fungi of aspargillosis *Aspergillus flavus* Link and *Aspergillus niger* Tieghem, yeast–like fungi *Candida albicans* and *Aurobasidium pullulans* [12, 36]. Thus, K.A. Aronstein and E. Saldivar [37] showed an increase in the profile of defensin expression level in the five-day old larvae *Apis mellifera* infected experimentally by *Ascosphaera apis*.

Protozoa. *Microsporidium nosema* is an obligate parasite in the honeybee intestine. *Nosema ceranae* was known until recently as the parasite of the China wax honeybee *A. cerana*. The data appeared from 2005 year about the parasitizing on *A. mellifera* of *N. ceranae* [38], which is more pathogenic for the honeybee, as compared with *Nosema apis* [39]. Possibly, this is a consequence of the long-term coevolution of *A. mellifera* with *N. apis,* promoting to development of certain immune mechanisms in honeybee to this parasite, also including the *defensin* gene expression [40]. *N. ceranae* is a new parasite for honeybee by producing the stronger disturbances in intestine by suppressing immunity in general and the defensin expression in particular.

Mites and viruses. A decrease of immunity by ectoparasites is due not only to the parasitizing itself, but also to transfer of viral infections. Mites are the most widely known ectoparasites of honeybees. Among many mite species, four species are the most dangerous for honeybees: *Varroa destructor, V. jarabsoni, Acarapis woodi, and Tropilaelaps clareae* [41]. The honeybees infected by *V. destructor* are known to die under the effect of bacteria *Esherichia coli* and differ from the health individuals by the higher number of destructed hemolymph cells and by their content of virus particles [41]. It has been shown that the immuno-

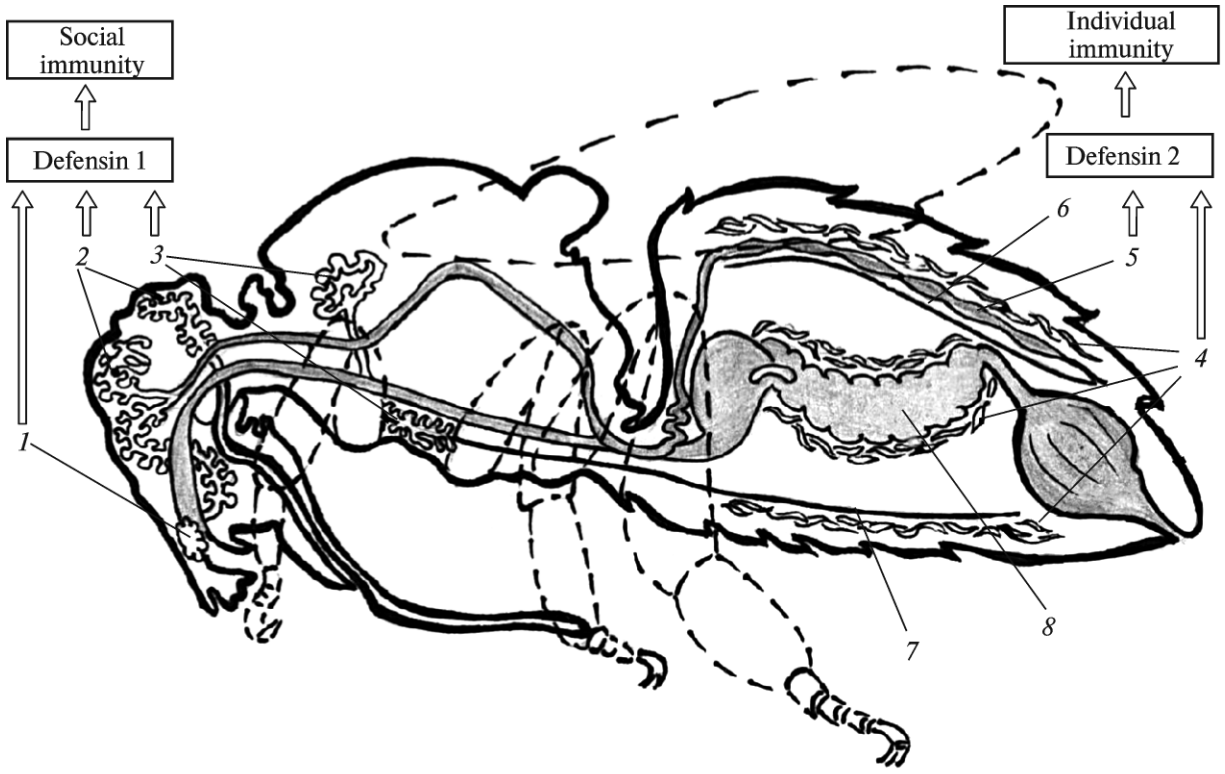

Fig. 3. The defensin forms in the honeybee individual and social immunity. (*1*) mandibular gland; (*2*) hypopharyngeal gland; (*3*) thoracic gland; (*4*) fat body; (*5*) dorsal vessel; (*6*) diaphragm; (*7*) abdominal diaphragm; (*8*) middle intestine.

suppressor effect and severe clinical signs rise with increase of the mite infection level [43].

V. destructor has a negative effect on the honeybee humoral immunity by decreasing level of the defensin gene transcription [42, 44]. Possibly, *V. destructor* produces the honeybee immunosuppression by replication of the deformation of the wing virus DWV, which is its carrier [45].

INDUCTION AND MECHANISMS OF ANTIMICROBIAL EFFECTS OF DEFENSINS

The majority of AMP are produced by cells of the fat body (adipocytes) and of hemolymph (hemocytes) during infections and disturbances of integument and are usually released into the hemolymph plasma [3, 46]. By using RT-PCR method it has been shown that defensin 1 is expressed in the honeybee head and thorax by the hypopharyngeal, mandibular, and thoracic glands [17] and is secreted into royal jelly [47] and honey [18]. Thus, it can be concluded about the predominant role of defensin 1 in development of the honeybee social immunity, unlike defensin 2 secreted by adipocytes and hemocytes and being a component of the individual immunity. The large representation of defensins in the honeybee organism indicates their universal and significant functions in the individual and social immunity.

There exists a wide variability of the amount and activity level of defensins in royal jelly in different healthy honeybee colonies. One of the causes can be the genetic variability in the defensin gene expression. The polymorphism of the defensin 1 gene can play an essential role in the AMP expression and in manifestation of its antimicrobial and antifungal activity [11]. The data of the study can be used for practical apiculture in selection of families more resistant to microbial pathogens [48]. The low level of transcription in norm and the presence of regulatory elements in the promoter region of defensin 2 gene prove that the peptide is induced by the pathogenic factor [1, 17]. Thus, defensin 2 is responsible for the honeybee individual immunity (Fig. 3).

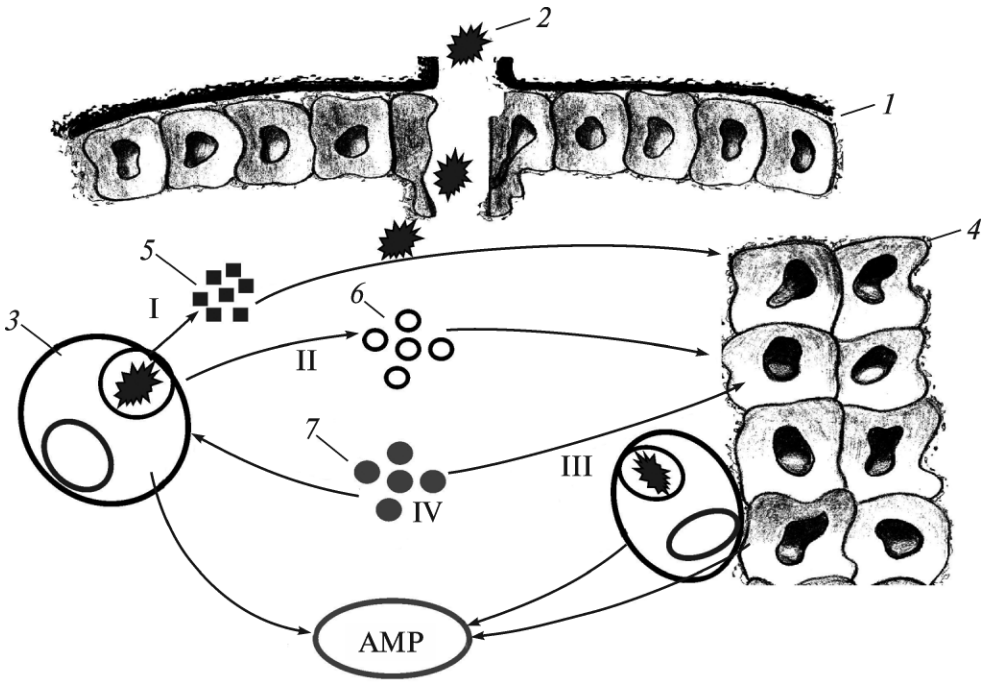

Fig. 4. Mechanisms of activation of AMP synthesis in the insect hemolymph and fat body. I—Generation of induction of AMP synthesis by phagocytes; II—secretion of the hemocyte transmitters; III—the phagocyte attachment to the fat body; IV—the endocrine regulation of AMP synthesis. (*1*) Integuments; (*2*) microorganism; (*3*) phagocyte; (*4*) adipocyte; (*5*) components of the microorganism cell walls; (*6*) the hemocyte transmitters; (*7*) transmitters of the endocrine system.

The portion of AMP synthesized by hemocytes is insignificant. The main part of AMP is produced by adipocytes [4]. The initiation of the transcriptional activity of AMP genes occurs during wound and entrance into hemolymph of different by their origin and nature inductors—bacteria, fungi, fragments of peptidoglycans and lypopolysaccharides of the bacteria cellular walls [49], some insecticides [50], and chitin oligomers [34, 35, 51]. Activation of the AMP synthesis in adipocytes and hemocytes is realized by several mechanisms (Fig. 4). It is suggested that phagocyte cells participate in generation of inductors of AMP synthesis—LPS, peptidoglycans, and β-1,3-glycans—secreting into hemolymph of components of cell walls of absorbed and digested bacteria [52] (Fig. 4, I). The activity of adipocytes and hemocytes can be enhanced by the hemocytic transmitters whose role can be played by prostaglandins [53] (Fig. 4, II). The phagocytes with absorbed microorganisms have been shown to be capable of attaching directly to cells of the fat body, which also stimulates the AMP synthesis [54] (Fig. 4, III). Besides, there is believed activation of the AMP synthesis in adipocytes and hemocytes under effect of transmitters of the endocrine system [55] (Fig. 4, IV).

In insects there exist two main NF-kB-mediated signal ways—the Imd and Toll ways controlling the AMP gene expression [55, 56]. The Toll way is responsible for protection against fungi and gram-positive bacteria, whereas the Imd way provides mainly protection against gram-negative bacteria [48]. At the same time, control of the defensin gene expression is realized by interaction of the Imd and Toll ways [36], which indicates importance of this element of the AMP system. AMP are active at a low concentration and show the wide specter of activity by interacting with membranes of the cell walls of pathogens and acting together with other agents of the immune response [56].

The general principle of AMP action not only is reduced to disturbance of integrity of membrane of pathogenic cells, but is also mediated by other mechanisms. The defensins kill the pathogenic cells by penetrating across their cytoplasm membrane [57]. The defensins produce formation of channels in the plasma membrane of cells-targets, through which the cytoplasmic K^+ outflows, and,

as a result, produces a decrease of ANP in the cytoplasm and inhibition of respiration processes. The experiments in vitro have shown defensins of Phormia and Aeschna also to produce a disturbance of the membrane permeability of sporozoites *Plasmodium gallinaceum* with a subsequent change in morphology and the loss of the parasite mobility [58].

CONCLUSION

The honeybee defensins have a high level of polymorphism and exist in two forms—defensins 1 and 2. The defensin gene expression is inducible and is controlled by the Imd and Toll signal ways, which provide universality of its effect against pathogens of honeybee (*Paenibacillus larvae, Melissococcus pluton, Ascosphaera apis, Nosema apis)*. The universality of this peptide of hymenoptera among other arthropods, as well as polyvariantness of antimicrobial activity of the honeybee defensins are due to variability of processing of the defensin precursors by leading to the appearance of multiple molecular forms. The mechanism of the defensin effect is reduced to disturbance of integrity and permeability of the cytoplasma membrane of pathogenic organisms and provides the wide specter of action.

The defensin 1 present in the royal jelly, honey, and salivary glands characterizes the social immunity of the colony on the whole, whereas the defensin 2 synthesized by cells of the fat body and hemolymph is responsible for the honeybee individual immunity.

ACKNOWLEDGMENTS

This work is supported by the Russian Foundation for Basic Research (projects nos. 08-04-97039-r povolzhie region-a and 11-04-97078-a).

REFERENCES

1. Casteels, P., Ampe, C., Jacobs, E., and Tempst, P., Functional and Chemical Characterization of Hymenoptaecin, an Antibacterial Polypeptide That Is Infection-Inducible in the Honeybee (*Apis mellifera*), *J. Biol. Chem.*, 1993, vol. 268, pp. 7044–7054.
2. Cremer, S., Armitage, S.A., and Schmid-Hempel, P., Social Immunity, *Curr. Biol.*, 2007, vol. 17, no. 16, pp. 693–702.
3. Hoffmann, J.A., Kafatos, F.C., Janawey, C.A., and Ezekovits, R.A.B., Phylogenetic Perspectives in Innate Immunity, *Science,* 1999, vol.284, pp. 1313–1318.
4. Hoffmann, J.A. and Richhart, J.-M., Drosophila Immunity, *Trends Cell Biol.*, 1997, vol. 7, pp. 309–316.
5. Brey, P.T., Lee, W., Yamakawa, M., Koizumi, Z., Perrot, S., Francois, M., and Ashida, M., Role of the Integument in Insect Immunity: Epicuticular Abrasion and Induction of Cecropin Synthesis in Cuticular Epithelial Cells, *Proc. Nat. Acad. Sci. USA,* 1993, vol. 90, pp. 6275–6279.
6. Lehane, M.J., Wu, D., and Lehane, S.M., Midgut-Specific Immune Molecules are Produced by the Blood-Sucking Insect *Stomoxys calcitrans*, *Proc. Nat. Acad. Sci. USA,* 1997, vol. 94, pp. 11502–11507.
7. Lowenberger, C.A., Smartt, C.T., Bulet, P., Ferdig, M.T., Severson, D.W., Hoffman, J.A., and Christensen, B.M., Insect Immunity: Molecular Cloning, Expression, and Characterization of cDNAs and Genomic DNA Encoding Three Isoforms of Insect Defensin in *Aedes aegypti*, *Insect Mol. Biol.*, 1999, vol. 8, pp. 107–118.
8. Aerts, A.M., Francois, I.E., Cammue, B.P., and Thevissen, K., The Mode of Antifungal Action of Plant, Insect and Human Defensins, *Cell. Mol. Life Sci.*, 2008, vol. 65, pp. 2069–2079.
9. Bulet, P., Hetru, C., Dimarcq, J.L., and Hoffmann, D., Antimicrobial Peptides in Insects; Structure and Function, *Dev. Comp. Immunol.*, 1999, vol. 23, pp. 329–344.
10. Miyagi, T., Peng, Ch.Y.S., Chuang, R.Y., Mussen, E.C., Spivak, M.S., and Doi, R.H., Verification of Oxytetracycline-Resistant American Foulbrood Pathogen Paenibacillus Larvae in the United States, *J. Invertebr. Pathol.*, 2000, vol. 75, pp. 95–96.
11. Bilikova, K., Gusui, W., and Simuth, J., Isolation of a Peptide Fraction from Honeybee Royal Jelly as a Potential Antifoulbrood Factor, *Apidologie,* 2001, vol. 32, pp. 275–283.
12. Chernysh, S.I., Gordya, N.A., and Filatova, N.A., Protective Mechanisms of Insects: the Temps of Molecular and Phenotypic Evolution, *Issled. Genet.,* 1999, Iss. 12, pp. 52–59.
13. Klaudiny, J., Hanes, J., Kulifajova, J., Albert, S., and Simuth, J., Molecular Cloning of Two cDNAs from the Head of the Nurse Honeybee (*Apis mellifera* L.) for Coding Related Proteins of Royal Jelly, *J. Apic. Res.*, 1994, vol. 33, pp. 105–111.
14. Casteels, P., Ampe, C., Jacobs, F., Vaek, M.,

and Tempst, P., Apidaecins: Antibacterial Peptides from Honeybees, *EMBO J.,* 1989, vol. 8, pp. 2387–2391.
15. Casteels, P., Ampe, C., Riviere, L., Damme, J.V., Elicone, C., Fleming, M., Jacobs, F., and Tempst, P., Isolation and Characterization of Abaecin, a Major Antibacterial Peptide in the Honeybee (*Apis mellifera*), *Eur. J. Biochem.*, 1990, vol. 187, pp. 381–386.
16. Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T., and Kobayashi, K.A., Potent Antibacterial Protein in Royal Jelly, *J. Biol. Chem.*, 1990, vol. 265, pp. 11 333–11 337.
17. Klaudiny, J., Albert, S., Bachanova, K., Kopernicky, J., and Simuth, J., Two Structurally Different Defensin Genes, One of them Encoding a Novel Defensin Isoform, are Expressed in Honeybee *Apis mellifera*, *Insect Biochem. Molec. Biol.,* 2005, vol. 35, pp. 11–22.
18. Kwakman, P.H.S., te Velde, A.A., de Boer, L., Speijer, D., Vandenbroucke-Grauls, C.M.J.E., and Zaat, S.A.J., How Honey Kills Bacteria, *The FASEB J.,* 2010, vol. 24, no. 7, pp. 2576–2582.
19. Casteels-Josson, K., Zhang, W., Capaci, T., Casteels, P., and Tempst, P., Acute Transcriptional Response of the Honeybee Peptide-Antibiotics Gene Repertoire and Required Posttranslational Conversion of the Precursor Structures, *J. Biol. Chem.*, 1994, vol. 269, pp. 28569–28575.
20. Hanzawa, H., Shimada, I., Kuzuhara, T., Komano, H., Kohda, D., Inagaki, F., Natori, S., and Arata, Y., 1H Nuclear Magnetic Resonance Study of the Solution Conformation of an Antibacterial Protein, Sapecin, *FEBS Lett.*, 1990, vol. 269, pp. 413–420.
21. Raj, P.A. and Dentino, A.R., Current Status of Defensins and Their Role in Innate and Adaptive Immunity, *FEMS Microbial. Lett.*, 2002, vol. 206, pp. 9–18.
22. Long, M., Evolution of Novel Genes, *Curr. Opinion Genet. Dev.*, 2001, vol. 11, pp. 673–680.
23. Rees, J.A., Moniatte, M., and Bulet, P., Novel Antibacterial Peptides Isolated from a European Bumblebee, *Bombus pacuorum* (Hymenoptera, Apoidea), *Insect Biochem. Mol. Biol.*, 1997, vol. 27, pp. 413–422.
24. Cornet, B., Bonmatin, J.-M., Hetru, C., Hoffmann, J.A., Ptak, M., and Vovelle, F., Refined Three-Dimensional Solution Structure of Insect Defensin A, *Structure,* 1995, vol. 3, pp. 435–441.
25. Yoshiyama, M. and Kimura, K., Characterization of Antimicrobial Peptide Genes from Japanese Honeybee *Apis cerana japonica* (Hymenoptera: Apidae), *Appl. Entomol. Zool.*, 2010, vol. 45, no. 4, pp. 609–614.
26. Ilyasov, R.A., Poskryakov, A.V., and Nikolenko, A.G., Polymorphism of the Antibacterial Preparations in the population of Ural Honeybees, *Bioraznoobrazie: problemy i perspektivy sokhraneniya* (Biovariety: Problems and Perspectives of Preservation), *Proc. Internat. Scient. Conference,* Penza, 2008, vol. 2, pp. 247–248.
27. Solbrig, O. and Solbrig, D., *Populyatsionnaya biologiya i evolutsiya* (Populational Biology and Evolution), Mir, Moscow, 1982, 244 pp.
28. Dimarcq, J.L., Hoffman, D., Meister, M., Bulet, P., Lanot, R., Reichhart, J.M., and Hoffman, J.A., Characterization and Transcriptional Profiles of a Drosophila Gene Encoding an Insect Defensin, *Eur. J. Biochem.*, 1994, vol. 221, pp. 201–209.
29. Lopez, L., Morales, G., Ursic, R., Wolff, M., and Lowneberger, C., Isolation and Characterization of a Novel Insect Defensin from *Rhodnius prolixus*, a Vector of Chagas Disease, *Insect Biochem. Mol. Biol.*, 2003, vol. 33, pp. 439–447.
30. Bulet, P. and Stocklin, R., Insect Antimicrobial Peptides: Structure, Properties and Gene Regulation, *Prot. Peptide Lett.*, 2005, vol. 12, pp. 3–11.
31. Arbia, K.A. and Babbay, B., Management Strategies of Honeybee diseases, *J. Entomol.*, 2011, vol. 8, no. 1, pp. 1–15.
32. Bachanova, K., Klaudiny, J., Kopernicky, J., and Simuth, J., Identifcation of Honeybee Peptide Active against *Paenibacillus larvae larvae* through Bacterial Growth–Inhibition Assay on Polyacrylamide Gel, *Apidologie*, 2002, vol. 33, pp. 259–269.
33. Yoon, H.J., Sohn, M.R., Young, M.C., Jianhong, L., Hung, D.S., and Byung, R.J., Defensin Gene Sequences of Three Different Bumblebees, Bombus spp., *J. Asia-Pacific Entomol.*, 2009, vol. 12, pp. 27–31.
34. Saltykova, E.S., Gaifullina, L.R., Ilyasov, R.A., and Nikolaenko, A.G., Effect of Chitozan on Induction of the Main Honeybee Antibacterial Peptides, *Sovremennye perspektivy v issledovanii khitina i khitozana* (Current Perspectives in the Study of Chitin and Chitozan), *Proc. Tenth Scient. Internat. Confer.,* Nizhnii Novgorod, 2010, pp. 308–310.
35. Saltyikova, E.S., Ilyasov, R.A., Gaifullina, L.R., Poskryakov, A.V., Yamidanov, R.S., and Nikolaenko, A.G., Change of the Level of Antibacterial Peptides in the Organism of Honeybee *Apis mellifera mellifera* L. , *Sovremennoe pchelovodstvo. Problemy, opyt, novye tekhnologii* (Current Apiculture. Problems, Experience, New Technologies), *Proc. Internat. Scient. Confer.,* Yaroslavl, 2010, pp. 159–160.

36. Aronstein, K.A., Murray, K.D., and Saldivar, E., Transcriptional Responses in Honeybee Larvae Infected with Chalkbrood Fungus, *BMC Genom.*, 2010, vol. 11, pp. 1–12.
37. Aronstein, K.A. and Saldivar, E., Characterization of a Honeybee Toll Related Receptor Gene Am18w and its Potential Involvement in Antimicrobial Immune Defense, *Apidologie*, 2005, vol. 36, pp. 3–14.
38. Higes, M., Martin-Hernandez, R., Gonzalez-Porto, A.V., Garcia-Palencia, P., Meana, A., and del Nozal, M.J., Honeybee Colony Collapse Due to *Nosema cernae* in Professional Apiaries, *Environ. Microbiol. Rep.*, 2009, vol. 1, pp. 110–113.
39. Klee, J., Besana, A.M., Genersch, E., Gisder, S., Nanetti, A., and Tam, D.Q., Widespread Dispersal of the Microsporidian *Nosema ceranae*, an Emergent Pathogen of the Western Honeybee, *Apis mellifera*, *J. Invertebr. Pathol.*, 2007, vol. 96, pp. 1–10.
40. Antunez, K., Martin-Hernandez, R., Prieto, L., Meana, A., Zunino, P., and Higes, M., Immune Suppression in the Honeybee (*Apis mellifera*) Following Infection by *Nosema ceranae* (Microsporidia), *Environ. Microbiol.*, 2009, vol. 11, no. 9, pp. 2284–2290.
41. Grobov, O.F. and Likhotin, A.K, *Bolezni i vrediteli pchel* (Diseases and Pests of Honeybees), Agropromizdat, Moscow, 1989, 239 p.
42. Yang, D., Biragyn, A., Hoover, D.M., Lubkowski, J., and Oppenheim, J.J., Multiple Roles of Antimicrobial Defensins, Cathelicidins, and Eosinophil-Derived Neurotoxin in Host Defense II *Annu. Rev. Immunol.* 2004, vol. 22, pp. 181–215.
43. Williams, G.R., Rogers, R.L., Kalkstein, A.L., Taylor, B.A., Shutler, D., and Ostiguy, N., Deformed Wing Virus in Western Honeybees (*Apis mellifera*) from Atlantic Canada, the First Description of an Overtlyinfected Emerging Queen, *J. Invertebr. Pathol.*, 2009, vol. 101, pp. 77–79.
44. Gregory, P.G., Evans, J.D., Rinderer, T., and de Guzman, L., Conditional Immune-Gene Suppression of Honeybees Parasitized by Varroa mites, *J. Insect Sci.*, 2005, vol. 5, pp. 1–5.
45. Genersch, E. and Aubert, M., Emerging and Re-Emerging Viruses of the Honeybee (*Apis mellifera* L.), *Vet. Res.*, 2010, vol. 41, no. 6, pp. 54–74.
46. Choi, Y.S., Choo, Y. M., Lee, K.S., Yoon, H.J., Kim, I., Je, Y.H., Sohn, H.D., and Jin, B.R., Cloning and Expression Profiling of Four Antibacterial Peptide Genes from the Bumblebee *Bombus ignites*, *Comp. Biochem. Physiol.,* 2008, vol. 150, pp. 141–146.
47. Qu, N., Jiang, J., Sun, L., Lai, C., Sun, L., and Wu, X., Proteomic Characterization of Royal Jelly Proteins in Chinese (*Apis cerana cerana*), European (*Apis mellifera*) Honeybees, *Biochemistry,* 2008, vol. 1, pp. 1–12.
48. Evans, J.D. and Spivak, M., Socialized Medicine Individual and Communal Disease Barriers in Honeybees, *J. Invertebr. Pathol.*, 2010, vol. 103, pp. 562–572.
49. Dunn, P.E., Humoral Immunity in Insects. Immune Strategy Appears to Correspond to Life-History Characteristics, *Biosci.,* 1990, vol. 40, no. 10, pp. 738–744.
50. Zhu, P. and Lu, Z., Studies on the Antibacterial Substances of *Pieris rapae* Induced by Deltamethrin and Trichlorfon, *19 Int. Congr. Entomol.*, Beijing, 1992, p. 594.
51. Furukawa, S., Taniai, K., Yang, J., Shono, T., and Yamakawa, M., Induction of Gene Expression of Antibacterial Proteins by Chitin Oligomers in the Silkworm, *Bombyx mori*, *Insect Molec. Biol.*, 1999, vol. 8, no. 1, pp. 145–148.
52. Taniani, K., Wago, H., and Yamakawa, M., In Vitro Phagocytosis of *Escherichia coli* and Release of Lipopolysaccharide by Adhering Hemocytes of the Silkworm, *Bombyx mori*, *Biochem. Biophys. Res. Commun.*, 1997, vol. 231, pp. 623–627.
53. Stanley-Samuelson, D.W., Prostaglandins, Related Eicosanoids in Insects, *Adv. Insect Physiol.*, 1994, vol. 24, pp. 115–212.
54. Faye, I. and Wyatt, G.R., The Synthesis of Antibacterial Proteins in Isolated Fat Body from Cecropia silkmoth Pupae, *Experientia*, 1980, vol. 36, pp. 1325–1326.
55. Glupov, V.V., *Patogeny nasekomykh: strukturnye i funktsionalnye aspekty* (Insect Pathogens: Structural and Functional Aspects), *Kruglyi God*, Moscow, 2001,736 p.
56. Osta, M.A., Christophides, G.K., Vlachou, D., and Kafatos, F.C., Innate Immunity in the Malaria Vector *Anopheles gambiae*: Comparative and Functional Genomics, *J. Exp. Biol.*, 2004, vol. 207, pp. 2551–2563.
57. Cociancich, S., Ghazi, A., Hetru, C., Hoffmann, J.A., and Letellier, L., Insect Defensin, an Inducible Antibacterial Peptide, Forms Voltage-Dependent Channels in *Micrococcus luteus*, *J. Biol. Chem.,* 1993, vol. 268, pp. 19239–19245.
58. Shahabuddin, M., Fields, I., Bulet, P., Hoffmann, J.A., and Miller, L., *Plasmodium gallinaceum*: Differential Killing of some Mosquito Stages of the Parasite by Insect Defensin, *Exper. Parasitol.*, 1998, vol. 89, no. 1, pp. 103–112.