Article

Transduction of the vitellogenic signal of juvenile hormone by Methoprene-tolerant in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae)

Mahboubeh Naghdi[1], **Jose-Luis Maestro**[2], **Xavier Belles**[2], **Ali Bandani**[1]

[1]Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, 31587-77871, Iran

[2]Institute of Evolutionary Biology (CSIC-Universitat Pompeu Fabra), 08003 Barcelona, Spain

E-mail: xavier.belles@ibe.upf-csic.es, abandani@ut.ac.ir

Received 27 June 2016; *Accepted 5 July 2016*; *Published 1 December 2016*

Abstract

In adult females of the cockroach *Blattella germanica*, juvenile hormone (JH) promotes vitellogenin production. Depletion of Methoprene-tolerant (Met) mRNA levels with RNAi resulted in a clear reduction of vitellogenin expression in the fat body while basal oocyte growth was impaired. This demonstrates that Met is essential to transduce the vitellogenic signal of JH in this species. Interestingly, the expression of the transcription factor Kruppel homolog 1 (Kr-h1) was also reduced in Met-depleted specimens. This indicates that the JH signaling pathway promotes the transcription of Kr-h1 in adult fat body tissues, as occurs in the epidermis during nymphal development. Treatments with JH show that the expression of Met does not depend on JH, which suggests that Met is upstream the JH signaling pathway acting as JH receptor, as reported in other models and processes, especially in metamorphosis. JH treatments increased the transcription levels of vitellogenin and Kr-h1, which again suggests that Kr-h1 is a JH-dependent transcription factor in the fat body of adult females. The important roles of Met in nymphal development, as previously reported, and those reported herein in relation to reproduction, suggests that it can be an interesting target for the control of *B. germanica* in urban environments, using RNAi approaches.

Keywords German cockroach; *Blattella*; vitellogenesis; juvenile hormone; methoprene-tolerant.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

In most insect species, especially in those phylogenetically more basal, vitellogenin production and oocyte growth is a JH-dependent process. This is the case, for example, of the cockroach *Blattella germanica*, in which a single vitellogenin has been characterized biochemically (Martin et al., 1995) and molecularly (Martin et al., 1998). In *B. germanica*, the pattern of vitellogenin production in the fat body of adult females (Martin et

al., 1995; Martin et al., 1998) coincides with the pattern of JH titer in the hemolymph (Treiblmayr et al., 2006). Moreover, JH treatment triggers a fast induction of vitellogenin expression *in vivo* (Comas et al., 1999) and *in vitro* (Comas et al., 2001). Nevertheless, nothing is known about the mechanisms of transduction of the vitellogenic signal of JH in this cockroach.

Recent studies have unveiled the identity of the bHLH-PAS protein Methoprene-tolerant (Met) as the JH receptor (Jindra et al., 2015b), especially in the context of insect metamorphosis (Jindra et al., 2015a; Jindra et al., 2013). However, the involvement of Met in the transduction of the vitellogenic signal of JH has been less documented. Among the hemimetabolan species where vitellogenin production is genuinely JH-dependent (Belles, 2005, Raikhel et al., 2004), the involvement of Met in vitellogenesis and oocyte maturation has been shown in the linden bug *Pyrrhocoris apterus* (Smykal et al., 2014), the migratory locust *Locusta migratoria* (Song et al., 2014) and the cockroach *Diploptera punctata* (Marchal et al., 2014). In holometabolian insects, the role of Met in reproductive processes has been shown in the fruitfly *Drosophila melanogaster*, where Met and germ cell-expressed (gce) mutants experienced retarded ovarian development and lower fecundity than wild type flies (Abdou et al., 2011; Wilson and Fabian, 1986). Similar results have been obtained in the mosquito *Aedes aegypti* after depleting Met mRNA levels by RNAi (Zou et al., 2013; Li et al., 2011). In the red flour beetle *Tribolium castaneum*, oogenesis is also impaired after Met depletion by RNAi (Parthasarathy et al., 2010). Notably, Met triggers vitellogenin expression in this beetle by acting upstream of insulin signaling; expression of the insulin-like peptide 2 in the brain and fat body requires JH and Met, and depletion of either the hormone or its receptor impairs vitellogenesis, but the effects can be counteracted with a bovine insulin treatment (Sheng et al., 2011).

In the present work, we have studied the effects of Met depletion by RNA interference (RNAi) in vitellogenesis and basal oocyte development in the German cockroach, *B. germanica*. The German cockroach is a widespread and serious urban pest, as it produces a number of proteins that can trigger allergic episodes that correlate with asthma development, and early exposure leads to increased sensitization and asthma severity (Dillon et al., 2015; Rosenstreich et al., 1997; Gore and Schal, 2007). Moreover, *B. germanica* is a potential vector of pathogenic and antibiotic-resistant microorganisms (Cloarec et al., 1992; Jalil et al., 2012; Zurek and Schal, 2004). Chemical insecticides are the most used tools for controlling cockroach populations in human habitats, although there are evidences showing that apartments treated with chemical pesticides and untreated apartments may support equal cockroach populations (Koehler et al., 1987; Schal and Hamilton, 1990). This illustrates the need to reexamine the current treatments that rely on insecticides and explore other alternatives. RNAi has been considered as an interesting approach for pest control (Katoch et al., 2013; Scott et al., 2013), also in human environments (Zhu et al., 2016). The German cockroach has been proven to be especially sensitive to RNAi (Belles, 2010), and previous reports have shown that Met is crucial to regulate postembryonic development in this species (Lozano and Belles, 2014). The present report describes that RNAi depletion of Met impairs vitellogenesis and oocyte growth in *B. germanica*, which reinforces the interest of Met as a target for controlling this urban pest using RNAi strategies.

2 Materials and Methods

2.1 Insects

Specimens of *B. germanica* were obtained from a colony reared on dry dog food (Panlab 125C3) and water in the dark at 30 ± 1°C and 60-70% relative humidity. Virgin females were used for the study of gene expression levels during the first gonadotrophic cycle, except in the case of day 8, when mated females on the first day of oothecal transport were used. Abdominal fat bodies were dissected under saline solution from carbon dioxide-anesthetized animals. After dissection, tissues were immediately frozen in liquid nitrogen and stored at -80˚C.

2.2 RNA extraction, cDNA synthesis and real-time PCR analyses

The expression levels of the different genes studied were measured using quantitative real-time PCR (qRT-PCR) using cDNA prepared from abdominal fat body as previously described (Maestro et al., 2009). Briefly, fat body tissue adhered to the abdominal sternites was dissected out, and 1 µg of total RNA was used for reverse transcription. The absence of genomic contamination was confirmed using a control without reverse transcription. cDNA amplifications of Met, vitellogenin, Kruppel homolog 1 (Kr-h1) and Actin 5C were performed in triplicate, in a 20 µl final volume, using the primers detailed in Table 1 (Lozano and Belles, 2014, Suren-Castillo et al., 2012; Lozano and Belles, 2011). cDNA levels were quantified using iQ SYBR Green supermix (Bio-Rad) in an iQ cycler and iQ single color detection system (Bio-Rad), as previously described (Ons et al., 2015). Real-time data was collected through the iQ5 optical system software v.2.0 (BioRad).

2.3 RNA interference

RNAi *in vivo* in females of *B. germanica* was performed as previously described (Maestro and Belles, 2006, Ciudad et al., 2006). For Met RNAi, the 458 bp dsRNA fragment (dsMet) described by Lozano and Belles (2014) was used. Primers to prepare the corresponding dsRNA are indicated in Table 1. In all experiments, a heterologous 307-bp fragment from the polyhedrin of *Autographa californica* nucleopolyhedrovirus (dsMock) was used as control (Lozano and Belles, 2014). A dose of 2 µg of dsRNA diluted in sterile saline was injected into the abdomen of freshly emerged adult females, and a second 2-µg dose was injected three days later. Effects were examined on day 5 of adult stage.

Table 1 Primers used to detect transcript levels by qPCR in *Blattella germanica* tissues and to prepare the dsRNA for RNAi experiments on Met. Gene abbreviations: *Met* (*Methoprene-tolerant*), vg (vitellogenin), *Kr-h1* (Kruppel homolog 1).

Primer set	Forward primer (5'-3')	Reverse primer (5'-3')	Reference sequence
RNAi			
Met	GCAAATTGTATCCTTCATCTGC	TGACAGACTCGCGCTTTATG	HG965209
qRT-PCR			
Met	CTGTTGGGACATCAGCAGAA	GGCAGGTGATGGAGTGAAGT	HG965209
vg	CTGGGCATTTGACAACACAACAT	TTGAAGAGCTGCTGGAGAGTTTG	AJ005115
Kr-h1	GCGAGTATTGCAGCAAATCA	GGGACGTTCTTTCGTATGGA	HE575250
Actin 5c	AGCTTCCTGATGGTCAGGTGA	TGTCGGCAATTCCAGGGTACATGGT	AJ862721

2.4 Juvenile hormone treatments

JH treatments were performed to assess that Met expression is not JH-dependent. A dose of 10 µg of JH III (Sigma) diluted in analytical grade acetone at a concentration of 10 µg/µl was topically applied on the abdominal tergites of freshly emerged or 2- day-old adult females, to which wings had been cut. Controls were equivalently treated with acetone. Dissections for mRNA measurements were carried out 24 h later.

3 Results

3.1 Expression profiles of vitellogenin and Met

Transcript levels of vitellogenin and Met were measured in the fat body of adult females throughout the first reproductive cycle. Vitellogenin mRNA levels followed a pattern which paralleled that of circulating JH (Treiblmayr et al., 2006), showing low levels at the beginning of the cycle, a peak at day 6, and a subsequent

rapid decline (Fig. 1A). Conversely, mRNA levels of Met did not show significant changes through the reproductive cycle (Fig. 1A).

3.2 RNAi depletion of Met reduces vitellogenin expression

In order to study the role of Met in vitellogenesis, Met mRNA levels were depleted using systemic RNAi. A dose of 2 µg of dsMet was injected into the abdomen of freshly emerged adult females, and the treatment was repeated at adult day 3. Females equivalently treated with dsMock were used as controls. Met mRNA levels were examined in the fat body of dsMet-treated females on day 5, and results showed that they were 67% lower as average in comparison with dsMock-treated controls (Fig. 1B). Moreover, vitellogenin mRNA levels showed 53% reduction as average in the dsMet-treated group compared with the dsMock-treated controls. This was concomitant with a dramatic reduction of the basal oocyte length (Fig. 1B). Interestingly, mRNA levels of Kr-h1were also reduced (55% as average) in Met-depleted specimens (Fig. 1B).

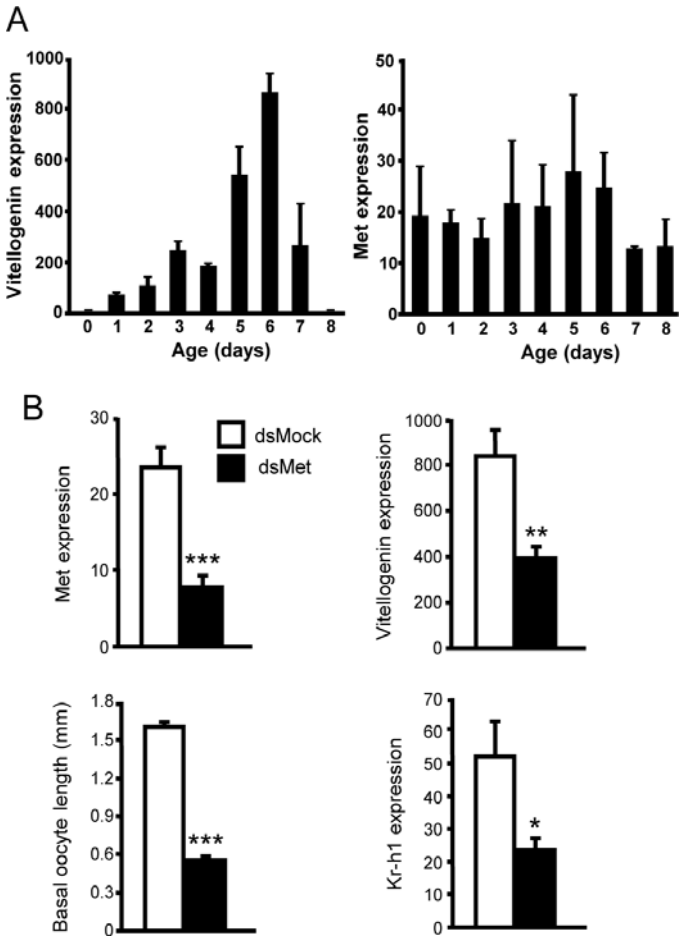

Fig. 1 Expression of vitellogenin and Methoprene-tolerant (Met) during the reproductive cycle of *Blattella germanica* and effect of RNAi treatment. A. Expression pattern of vitellogenin and Met in the fat body of adult females throughout the first reproductive cycle (n = 3); day 8 is the first day of ootheca transport. B. Effect of Met depletion in the fat body of adult females. Freshly ecdysed adult females were injected with dsMet or dsMock (Control) at days 0 and 3; fat body mRNA levels of Met, vitellogenin and Kr-h1, and basal oocyte length were measured at adult day 5 (n = 10-11 for mRNA measurements, and n = 16-19 for basal oocyte length). Results are expressed as the mean ± S.E. Gene expression is represented as copies of the given transcript per copy of Actin 5C in the case of vitellogenin, and copies per 1000 copies of Actin 5C for Met and Kr-h1 transcripts. Asterisks represent significant differences between control and dsMet (Student's *t* test, **P*<0.05; ***P*<0.001; ****P*<0.0001).

3.3 JH treatment does not affect Met expression

We aimed at assessing that Met is at the top of the JH signaling pathway in the regulation of vitellogenesis, as it is in the regulation in metamorphosis, and whose expression is not JH-dependent. Thus, we treated freshly emerged adult females with 10 µg of JH III and we quantified the expression of Met 24 h later. JH treatment did not affect Met expression, as expected (Fig. 2A). Conversely, it produced a dramatic increase of vitellogenin expression in fat body, and a more modest, although statistically significant, increase of Kr-h1 mRNA levels. When we carried out the same JH treatment but in 2 day old adult females, Met was again unaffected, whereas vitellogenin mRNA levels tended to increase and Kr-h1 mRNA levels significantly increased (Fig. 2B).

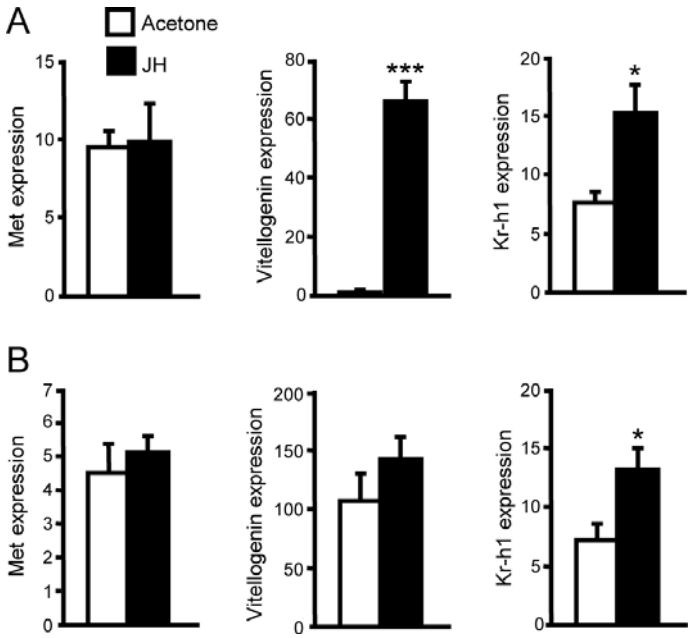

Fig. 2 Effects of exogenous juvenile hormone (JH) treatments on Methoprene-tolerant (Met) expression in *Blattella germanica*. Effects of treatment (10 µg of JH III or acetone) carried out on freshly ecdysed adult females and examined on day 1 on fat body tissues (n = 6). B. Effects of treatment (same dose and control) carried out on day 2 adult females and examined on day 3 on fat body tissues (n = 6). Results are expressed as the mean ± S.E. Gene expression is represented as copies of the given transcript per copy of Actin 5C in the case of vitellogenin, and copies per 1000 copies of Actin 5C for Met and Kr-h1 transcripts. Asterisks represent significant differences (Student's *t* test, **P*<0.05; ****P*<0.000).

4 Discussion

The pattern of vitellogenin transcription in the fat body of adult female *B. germanica* during the first reproductive cycle is similar to that obtained previously using semi-quantitative methods (Martin et al., 1998) and parallels the pattern of vitellogenin in terms of protein (Martin et al., 1995). These patterns are also coincident with that of JH titer in the hemolymph (Treiblmayr et al., 2006), which is not surprising, as vitellogenin expression in *B. germanica* is strictly JH-dependent (Comas et al., 1999; Comas et al., 2001).

Although the pattern of Met expression in the fat body do not correlate with that of vitellogenin expression, depletion of Met mRNA levels resulted in a clear reduction of vitellogenin expression in the fat body and of basal oocyte length. Interestingly, Kr-h1 expression was also reduced, which indicates that the JH signaling pathway promotes its transcription in adult fat body tissues, as occurs in nymphal epidermis in the context of nymphal development and metamorphosis (Lozano and Belles, 2011). Intriguingly, Met depletion in *P.*

apterus (Smykal et al., 2014) and *L. migratoria* (Song et al., 2014) resulted in relatively higher reductions of vitellogenin and Kr-h1 mRNA levels, as if vitellogenin and Kr-h1 expression were less sensitive to Met in *B. germanica*. In any case, the results suggest that Met is necessary to transduce the vitellogenic signal of JH in *B. germanica*. This is in agreement with other reports based on hemimetabolan (Marchal et al., 2014, Smykal et al., 2014, Song et al., 2014) and holometabolan (Abdou et al., 2011; Li et al., 2011; Wilson and Fabian, 1986; Zou et al., 2013) species. Thus, the function of Met as initial transductor of JH in relation to vitellogenesis and oocyte development, in general, appears conserved from cockroaches to flies.

The treatments with exogenous JH show that the expression of Met does not depend on JH, which suggests, as expected, that Met is upstream the JH signaling pathway acting as JH receptor, as showed in other models and processes, especially in the regulation of metamorphosis (Jindra et al., 2015a; Jindra et al., 2013). The increase of vitellogenin transcription after the JH treatment is not surprising as this has been previously reported (Comas et al., 1999; Comas et al., 2001) in *B. germanica*, and serves here as a positive control to show that the treatment was efficient, especially when JH was administered on freshly emerged females. The lower effect in the treatment on 2-day-old females is probably due to the high levels of vitellogenin transcription that occur at this age (Fig. 1A), which can be hardly increased even with exogenous JH. Interestingly, mRNA levels of Kr-h1 significantly increased after JH treatment at the two ages assayed. This again suggests that Kr-h1 is a JH-dependent transcription factor in the fat body of adult females.

A previous report had shown that RNAi of Met induces precocious metamorphosis, triggering the formation of non-viable miniature adults (Lozano and Belles, 2011). The present results demonstrate that Met depletion impairs vitellogenesis and basal oocyte growth. Thus, RNAi of Met affects nymphal development as well as adult reproduction, which makes it a potential target for German cockroach control using RNAi strategies.

Acknowledgements

This work was supported by the Spanish Ministerio de Economía y Competitividad (grants CGL2012-36251 and CGL2015-64727-P to X.B., and BFU2010-15906 to JLM), and Catalan Government (2014 SGR 619). The research has also benefited from FEDER funds. Thanks are also due to Maria-Dolors Piulachs for helpful discussions. MN was recipient of a grant from the University of Tehran to work in Belles lab, in Barcelona.

References

Abdou MA, He Q, Wen D, Zyaan O, Wang J, Xu J, Baumann AA, Joseph J, Wilson TG and Li, S. 2011. *Drosophila* Met and Gce are partially redundant in transducing juvenile hormone action. Insect Biochemistry and Molecular Biology, 41: 938-945

Belles X. 2005. Vitellogenesis directed by juvenile hormone. In: Progress in Vitellogenesis. Reproductive Biology of Invertebrates (Raikhel AS, Sappington TW, eds). 157-197, Science Publishers Inc, Enfield, USA

Belles X. 2010. Beyond *Drosophila*: RNAi in vivo and functional genomics in insects. Annual Review of Entomology, 55: 111-128

Ciudad L, Piulachs MD, Belles X. 2006. Systemic RNAi of the cockroach vitellogenin receptor results in a phenotype similar to that of the *Drosophila* yolkless mutant. FEBS Journal, 273 : 325-335

Cloarec A, Rivault C, Fontaine F, Le Guyader A. 1992. Cockroaches as carriers of bacteria in multi-family dwellings. Epidemiology and Infection, 109: 483-490

Comas D, Piulachs, MD, Belles X. 1999. Fast induction of vitellogenin gene expression by juvenile hormone III in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Insect Biochemistry and Molecular Biology, 29: 821-827

Comas D, Piulachs MD, Belles X. 2001. Induction of vitellogenin gene transcription in vitro by juvenile hormone in *Blattella germanica*. Molecular and Cellular Endocrinology, 183: 93-100

Dillon MB, Schulten V, Oseroff C, Paul S, Dullanty LM, Frazier A, Belles X, Piulachs MD, Visness C, Bacharier L, Bloomberg GR, Busse P, Sidney J, Peters B, Sette A. 2015. Different Bla-g T cell antigens dominate responses in asthma versus rhinitis subjects. Clinical and Experimental Allergy, 45: 1856-1867

Gore JC, Schal C. 2007. Cockroach allergen biology and mitigation in the indoor environment. Annual Review of Entomology, 52: 439-463

Jalil N, Amir K, Hasan MKS, Mahdi M, Monireh M, Atefeh B. 2012. Cockroaches' bacterial infections in wards of hospitals, Hamedan city, west of Iran. Asian Pacific Journal of Tropical Disease, 2: 381-384

Jindra M, Belles X, Shinoda T. 2015a. Molecular basis of juvenile hormone signaling. Current Opinion in Insect Science, 11: 39-46

Jindra M, Palli SR, Riddiford LM. 2013. The juvenile hormone signaling pathway in insect development. Annual Review of Entomology, 58: 181-204

Jindra M, Uhlirova M, Charles JP, Smykal V, Hill RJ. 2015b. Genetic Evidence for Function of the bHLH-PAS Protein Gce/Met As a Juvenile Hormone Receptor. PLoS Genetics, 11: e1005394

Katoch R, Sethi A, Thakur N, Murdock LL. 2013. RNAi for insect control: current perspective and future challenges. Applied Biochemistry and Biotechnology, 171: 847-873

Koehler PG, Patterson RS, Brenner RJ. 1987. German cockroach (Orthoptera: Blattellidae) infestations in low-income apartments. Journal of Economic Entomology, 80: 446-450

Li M, Mead EA, Zhu J. 2011. Heterodimer of two bHLH-PAS proteins mediates juvenile hormone-induced gene expression. Proceedings of the National Academy of Sciences USA, 108: 638-643

Lozano, J, Belles X. 2011. Conserved repressive function of Krüppel homolog 1 on insect metamorphosis in hemimetabolous and holometabolous species. Scientufuc Reports, 1: 163

Lozano J, Belles X. 2014. Role of Methoprene-tolerant (Met) in adult morphogenesis and in adult ecdysis of *Blattella germanica*. PLoS One, 9: e103614.

Maestro JL, Belles X. 2006. Silencing allatostatin expression using double-stranded RNA targeted to preproallatostatin mRNA in the German cockroach. Archives of Insect Biochemistry and Physiology, 62: 73-79

Maestro JL, Cobo J, Belles X. 2009. Target of rapamycin (TOR) mediates the transduction of nutritional signals into juvenile hormone production. Journal of Biological Chemistry, 284: 5506-5513

Marchal E, Hult EF, Huang J, Pang Z, Stay B, Tobe SS. 2014. Methoprene-tolerant (Met) knockdown in the adult female cockroach, *Diploptera punctata* completely inhibits ovarian development. PLoS One, 9: e106737

Martin D, Piulachs MD, Belles X. 1995. Patterns of haemolymph vitellogenin and ovarian vitellin in the German cockroach, and the role of juvenile hormone. Physiological Entomology, 20: 59-65

Martin D, Piulachs MD, Comas D, Belles X. 1998. Isolation and sequence of a partial vitellogenin cDNA from the cockroach, *Blattella germanica* (L.) (Dictyoptera, Blattellidae), and characterization of the vitellogenin gene expression. Archives of Insect Biochemistry and Physiology, 38: 137-146

Ons S, Belles X, Maestro JL. 2015. Orcokinins contribute to the regulation of vitellogenin transcription in the cockroach *Blattella germanica*. Journal of Insect Physiology, 82: 129-133

Parthasarathy R, Sun Z, Bai H, Palli SR. 2010. Juvenile hormone regulation of vitellogenin synthesis in the red

flour beetle, *Tribolium castaneum*. Insect Biochemistry and Molecular Biology, 40: 405-414

Raikhel AS, Brown MR, Belles X. 2004. Hormonal control of reproductive processes. In: Comprehensive Molecular Insect Science (Gilbert LI, Iatrou, K Gill SS, eds). 432-491, Elsevier, San Diego, USA

Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, Mitchell H, Mcniff-Mortimer K, Lynn H, Ownby D, Malveaux F. 1997. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. New England Journal of Medicine, 336: 1356-1363

Scott JG, Michel K, Bartholomay LC, Siegfried, BD, Hunter WB, Smagghe G, Zhu KY, Douglas AE. 2013. Towards the elements of successful insect RNAi. Journal of Insect Physiology, 59: 1212-1221

Schal C, Hamilton RL. 1990. Integrated suppression of synanthropic cockroaches. Annual Review of Entomology, 35: 521-551

Sheng Z, Xu J, Bai H, Zhu F, Palli SR. 2011. Juvenile hormone regulates vitellogenin gene expression through insulin-like peptide signaling pathway in the red flour beetle, *Tribolium castaneum*. Journal of Biological Chemistry, 286: 41924-41936

Smykal V, Bajgar A, Provaznik J, Fexova S, Buricova M, Takaki K, Hodkova, M, Jindra M, Dolezel D. 2014. Juvenile hormone signaling during reproduction and development of the linden bug, *Pyrrhocoris apterus*. Insect Biochemistry and Molecular Biology, 45: 69-76

Song J, Wu Z, Wang Z, Deng S, Zhou S. 2014. Kruppel-homolog 1 mediates juvenile hormone action to promote vitellogenesis and oocyte maturation in the migratory locust. Insect Biochemistry and Molecular Biology, 52: 94-101

Suren-Castillo S, Abrisqueta M, Maestro JL. 2012. FoxO inhibits juvenile hormone biosynthesis and vitellogenin production in the German cockroach. Insect Biochemistry and Molecular Biology, 42: 491-498

Treiblmayr K, Pascual N, Piulachs MD, Keller T, Belles X. 2006. Juvenile hormone titer versus juvenile hormone synthesis in female nymphs and adults of the German cockroach, *Blattella germanica*. Journal of Insect Science, 6: 1-7

Wilson TG, Fabian J. 1986. A *Drosophila melanogaster* mutant resistant to a chemical analog of juvenile hormone. Developmental Biology, 118: 190-201

Zhu F, Lavine L, O'neal S, Lavine M, Foss C, Walsh D. 2016. Insecticide Resistance and Management Strategies in Urban Ecosystems. Insects, 7: 1

Zou Z, Saha TT, Roy S, Shin SW, Backman TW, Girke T, White KP, Raikhel AS. 2013. Juvenile hormone and its receptor, methoprene-tolerant, control the dynamics of mosquito gene expression. Proceedings of the National Academy of Sciences USA, 110: 2173-2181

Zurek L, Schal C. 2004. Evaluation of the German cockroach (*Blattella germanica*) as a vector for verotoxigenic *Escherichia coli* F18 in confined swine production. Veterinary Microbiology, 101: 263-267