Isolation and Sequence of a Partial Vitellogenin cDNA From the Cockroach, *Blattella germanica* (L.) (Dictyoptera, Blattellidae), and Characterization of the Vitellogenin Gene Expression

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A partial cDNA clone of the vitellogenin gene from the cockroach *Blattella germanica* has been isolated from a cDNA expression library using an anti-vitellin–vitellogenin antiserum probe. The analysis of cDNA inserts gave a sequence of 2,645 nucleotides corresponding to the 3′ region. The deduced amino acid sequence is 825 residues long and is similar to the homologous portion of the vitellogenin of other insect species, especially that of the mosquito *Aedes aegypti*. RNA hybridization studies indicated that the vitellogenin gene expression is limited to the fat body of adult females. The pattern of expression during the first vitellogenic cycle was approximately parallel to that of vitellogenin production by the fat body previously described. The availability of a cDNA probe for the *B. germanica* vitellogenin gene represents a useful tool to study the molecular action of hormones affecting vitellogenin synthesis in this species. Arch. Insect Biochem. Physiol. 38:137–146, 1998. © 1998 Wiley-Liss, Inc.

Key words: insect, Dictyoptera, cockroach; *Blattella germanica*, vitellogenin

INTRODUCTION

In the cockroach *Blattella germanica*, the ootheca is carried externally throughout the developmental period of the eggs, and vitellogenesis occurs in discrete cycles separated by these periods of ootheca transport. Therefore, *B. germanica* represents an intermediate evolutionary step between strictly oviparous cockroaches, like *Periplaneta americana*, which drop the ootheca shortly after its formation, and viviparous species, like *Diploptera punctata*, which incubate the eggs in an internal brood sac (Roth, 1970). This makes *B. germanica* a good model not only to study the mechanisms that regulate vitellogenesis, but to elucidate possible evolutionary routes that resulted in viviparity in cockroaches as well.

In previous papers on *B. germanica* we have described that the patterns of production of juvenile hormone (Bellés et al., 1987; Maestro et al., 1994) and vitellogenin (Martín et al., 1995a,b, 1996) are cyclic and that both cycles are approximately parallel during the first days of adult life. This was not surprising given that juvenile hor-

mone is the main gonadotrophic factor in this species, as shown by experiments of allatectomy (Piulachs, 1988). However, toward the second half of the vitellogenic process, the cycle of juvenile hormone appears delayed with respect to that of vitellogenin (cf. Maestro et al., 1994; Martín et al., 1995b). This finding suggests that there must be a mechanism other than a simple decrease of juvenile hormone that governs the termination of vitellogenesis.

In order to initiate an analysis at molecular level, we describe the isolation and partial sequence of a vitellogenin cDNA and its use as a probe in Northern blot analysis to assess the developmental, tissue, and sex specificity of the vitellogenin gene expression in this cockroach. The deduced amino acid sequence of the *B. germanica* vitellogenin also allowed comparison with other species in which the homologous sequence has been reported, like the coleopteran *Anthonomus grandis* (Trewitt et al., 1992), the dipteran *Aedes aegypti* (Chen et al., 1994; Romans et al., 1995), the lepidopterans *Bombyx mori* (Yano et al., 1994a,b), and *Lymantria dispar* (Hiremath and Lehtoma, 1997a,b), and the hymenopterans *Pimpla nipponica* and *Athalia rosae* (Nose et al., 1997) (see Bellés, 1998, for review).

MATERIALS AND METHODS

Insect Rearing and Tissue Collection

Specimens of *B. germanica* were taken from a colony reared in the dark at 30 ± 1ºC and 60–70% relative humidity. Freshly molted adult virgin females were isolated and used at appropriate physiological ages, which were assessed by measuring the basal oocyte length. Fat bodies used to determine mRNA levels were dissected out by standard surgical methods, taking care to obtain the same proportion of tissue per specimen each time (ca. 90% of the abdominal fat body).

Construction of a cDNA Expression Library

Fat bodies from 55 females, between days 3 and 5 of adult life, were used as starting material. Total RNA was extracted by the phenol/chloroform method (Chomczynski and Sacchi, 1987). Poly(A$^+$) RNA was purified from total RNA by using the PolyATtract system (Promega, Madison, WI). A total of 5 μg of poly(A$^+$) RNA was used to construct a cDNA library in the ZAP cloning vector (UNI-ZAPXR synthesis kit, Stratagene, La Jolla, CA), using Gigapack II Gold packaging extracts (Stratagene), in all cases following the supplier's instructions. The library was amplified once before screening.

Isolation of the Vitellogenin cDNA Clones

The library was grown in 150-mm NZY medium plates at an initial density of 50,000 plaques per plate in the *Escherichia coli* XL1-blue MRF strain, transferred to nitrocellulose membranes (Hybond-C Extra, Amersham, Buckinghamshire, UK), impregnated with isopropyl-β-ᴅ-thiogalactopyranoside (IPTG), and incubated with the primary antibody, rabbit anti-vitellin–vitellogenin of *B. germanica*, described by Martín et al. (1995a). Enhanced chemilumescence (ECL) Western blotting (Amersham) was used as the detection system, following the manufacturer's protocol. After four successive screenings at low-density colonies, three putative clones for vitellogenin were isolated and sequenced.

DNA Sequence Analysis

Sequence analysis of the selected clones was directly performed after in vivo excision on the pBluescript vector by the dideoxynucleotide chain termination method (Sanger et al., 1977). Clones were sequenced on both strands using the T3 and T7 sequencing primers and then internal, specific primers, in the automated fluorescence sequencing system ABI (Perkin Elmer, Norwalk, CT). Oligonucleotides were synthesized on an Applied Biosystems 392 DNA synthesizer (Foster City, CA).

RNA Isolation and Nothern Blot Analysis

Total RNA was extracted from fat body from female last-instar nymphs, 5-day-old males, or adult females, following the phenol/chloroform method (Chomczynski and Sacchi, 1987). Total RNA (5 μg) was subjected to electrophoresis in 1.2% agarose gels containing formaldehyde and then transferred to nylon membranes (Hybond-N$^+$, Amersham). The isolated cDNA inserts used as probes were labeled with fluorescein using the Gene Images random prime labeling module (Amersham). Positive hybridizations were detected with the Gene Images CDP-Star detection module (Amersham) according to the supplier's protocol. Size of RNAs was estimated with RNA markers (USB, Amersham).

Sequence Comparisons and Analysis

Vitellogenin amino acid sequences used for comparison were obtained from literature sources and the GenBank database as follows: silkworm

moth, *B. mori* (Yano et al., 1994b; accession number D13160); gypsy moth, *L. dispar* (Hiremath and Lehtoma, 1997a; accession number V60186); boll weevil, *A. grandis* (Trewitt et al., 1992; accession number M72980); yellow fever mosquito, *A. aegypti* (Chen et al., 1994; accession number U02548); turnip sawfly, *Athalia rosae* (Nose et al., 1997; accession number AB007850); the parasitoid wasp *Pimpla nipponica* (Nose et al., 1997; accession number AF026789); and the nematode *Caenorhabditis elegans* (vit-5: Spieth et al., 1985; accession number X03044) as a non-insect invertebrate reference. The software package of the Genetics Computer Group (GCG, version 9.1) of the University of Wisconsin (Devereux et al., 1984) was used for sequence alignments. Sequence alignments were carried out with PILEUP and were displayed with BOX. Percentage of similarity and of identity between sequences was estimated with the application BESTFIT.

RESULTS

Isolation of Vitellogenin cDNA Clones

The cDNA library constructed contained 1×10^8 independent clones, and was screened with the anti-vitellin–vitellogenin antiserum. Approximately 4×10^5 plaques were screened and 40 positive clones were obtained. After four screening cycles, three positive clones were isolated and subjected to DNA sequence analysis. These clones were designed Vg8a, Vg3b, and Vg16a, and the approximate size of the cDNA inserts was 2,650, 2,100, and 1,700 base pairs (bp), respectively (Fig. 1). The restriction map, which was the same for all clones, and the strategy of nucleotide sequencing of these fragments of vitellogenin cDNA are shown in Figure 1.

Nucleotide and Amino Acid Sequences of Vitellogenin cDNA Clones

The nucleotide sequence corresponding to the longest cDNA clone (Vg8a) was 2,645 nucleotides long and revealed an open reading frame of 835 amino acid residues flanked by 3´ untranslated region of 139 bp (Fig. 2) (EMBL/GenBank database accession number: AJ005115). This open reading frame was chosen because the amino acid sequence inferred from it aligned well with other vitellogenins (see next section). The polyadenylation signal present in the 3´ flanking region begins at position 2599 and the poly(A) tail starts some 20 nucleotides down-
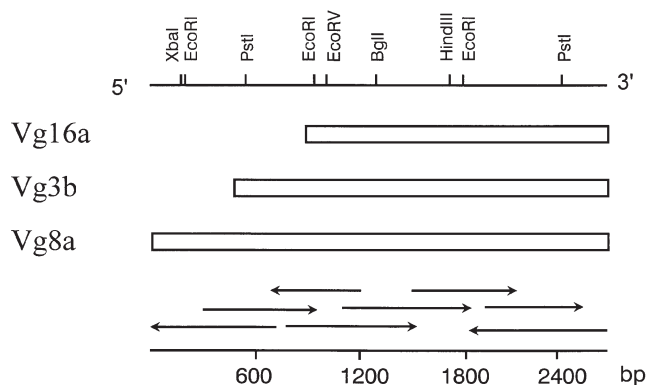

Fig. 1. Restriction map and sequencing strategies of cDNAs and vitellogenin mRNA of *Blattella germanica*. Open boxes, cDNA inserts in each clone. Arrows, strategy used to determine the nucleotide sequence.

stream of the polyadenylation signal. In the deduced amino acid sequence (Fig. 2), two clusters of serine residues occur at positions 664–675 and 701–711, and putative glycosylation sites are present at positions 89, 214, 298, 322, 382, 393, 404, 455, 539, 584, 679, 689, and 714. In addition, two putative cleavage sites for the protease of the subtilisin family (Barr, 1991) are located at positions 144 and 310. The nucleotide sequences of clones Vg16a and Vg3b were identical to the corresponding fragment of Vg8a (Fig. 1).

Comparison With Vitellogenin Sequences of Other Species

Comparison of the partial amino acid sequence of *B. germanica* (Fig. 2) with the homologous portion of *A. grandis*, *A. aegypti*, *B. mori*, *L. dispar*, *P. nipponica*, and *A. rosae* revealed that only the mosquito *A. aegypti* had a characteristic polyserine domain toward the 3´ end of the sequence. Therefore, a first alignment was carried out between the sequences of *B. germanica* and *A. aegypti*. The alignment (Fig. 3) displayed numerous conserved motifs (like the tetrapeptides TFDN and GLCG) which are typical of vitellogenin sequences. The percentages of similarity and identity between the sequences of the two species were 32% and 25%, respectively.

Given that the portion of the vitellogenin sequence available in *B. germanica* included the entire subdomain IV as defined by Chen et al. (1997), we aligned the sequence corresponding to this subdomain in all insects in which it was available and, as an external reference, vitellogenin 5 (vit 5) from the nematode *C. elegans* was also included. The results (Fig. 4) indicate a rela-

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   1  TTCGGCACGAGTCCATACACAACCATCCATAACGTACCAGACTTTGAAACAGTCTACATGGATCAACTCTTCAAA
   1  F  G  T  S  P  Y  T  T  I  H  N  V  P  D  F  E  T  V  Y  M  D  Q  L  F  K

  76  TTGATCCACGTGCGAAAAACAGCACACTTCGAGAAGAAAATGGGAGAAAACACGGGCATTGTATTTAAAGTAAAA
  26  L  I  H  V  R  K  T  A  H  F  E  K  K  M  G  E  N  T  G  I  V  F  K  V  K

 151  TATGATACAGATCAGGAATTTCTGGATACAAAGTGGTTTCTAGATGAATTCAAGGTTCTCCAACTTTTCACTGGT
  51  Y  D  T  D  Q  E  F  L  D  T  K  W  F  L  D  E  F  K  V  L  Q  L  F  T  G

 226  CTTAATTATGACGTGCCCACAAAGGACATCTTCTACAACAACCTGACTGTATATTATGATCATGAAGACACCAAA
  76  L  N  Y  D  V  P  T  K  D  I  F  Y  N  **N  L  T**  V  Y  Y  D  H  E  D  T  K

 301  AACCATGCCGTATCTTTTACTGTGACAAAAGAACAATCTAAGTTCTACGAAACATTAAACCCAGTAGTTCAACAA
 101  N  H  A  V  S  F  T  V  T  K  E  Q  S  K  F  Y  E  T  L  N  P  V  V  Q  Q

 376  AACCTAAAACTTTCATCTGGAAAGAAGCAAAAACATCGTAATGTAAAGAGCCATCGTATAAGGAGAGAATATACC
 126  N  L  K  L  S  S  G  K  K  Q  K  H  R  N  V  K  S  H  <u>R  I  R  R</u>  E  Y  T

 451  GAGGATGAAAATCCAGCTATTCCAAAAGACAAACAACCAAACAGCCATCCCAGACGTCAAGAATACCTAAGCAAA
 151  E  D  E  N  P  A  I  P  K  D  K  Q  P  N  S  H  P  R  R  Q  E  Y  L  S  K

 526  TCAATGGCACTAACTGGAGATGCAACTGCAGTTGTACTTGATATGACTTTGAAGTTTGAGGGGCCTGCTGAATCC
 176  S  M  A  L  T  G  D  A  T  A  V  V  L  D  M  T  L  K  F  E  G  P  A  E  S

 601  TACTTTACAACCACCGTATCTCACGCTACATCTCTTGTTAATGGTAGCTCTAACTACCTACTCTTCTACGATCAA
 201  Y  F  T  T  T  V  S  H  A  T  S  L  V  **N  G  S**  S  N  Y  L  L  F  Y  D  Q

 676  CACTACTATGAGGAAAAGAAGAGAAATCAGTTCTGTCTTTCATGGAGTGTCTACAAACCACAAGTTCCAATCATG
 226  H  Y  Y  E  E  K  K  R  N  Q  F  C  L  S  W  S  V  Y  K  P  Q  V  P  I  M

 751  AATATTTATAGTGCTTTTGAATTTGATCCAAATTCCAAAGTGCATGCCATAATGAACATAGGAAAAGAATGTGAG
 251  N  I  Y  S  A  F  E  F  D  P  N  S  K  V  H  A  I  M  N  I  G  K  E  C  E

 826  AATGGAGGAAGTGCTGTAGCAAATATTGATATGCTTAGATTGTCAGAACACCTAGATTACGTAAAGAATTTAACA
 276  N  G  G  S  A  V  A  N  I  D  M  L  R  L  S  E  H  L  D  Y  V  K  **N  L  T**

 901  GTCAGCAAGCTATGCGACCATGAAATGCGTACAAAACGTGATCACGTTCTGCCAGCTTGCAGGAATTCTACTGAG
 301  V  S  K  L  C  D  H  E  M  <u>R  T  K  R</u>  D  H  V  L  P  A  C  R  **N  S  T**  E

 976  AGAGCCAGTGATCTCAACAGAGTTCATGTTGATATCAATTATAACTTAAAGCAGCATGAAACATTCAAACGCAGA
 326  R  A  S  D  L  N  R  V  H  V  D  I  N  Y  N  L  K  Q  H  E  T  F  K  R  R

1051  GTATACAAAGTATATGATTTTGTACGTACCCACCTCTATCCACACGTATCAGAGGATGTGATCGTTGATAATCCA
 351  V  Y  K  V  Y  D  F  V  R  T  H  L  Y  P  H  V  S  E  D  V  I  V  D  N  P

1126  GCACAATTCATATCGGCAAATTTCACATTGAAAGACAATACAAGAGCCTTCAACGTGAGCATTGAAACTCCTGTA
 376  A  Q  F  I  S  A  **N  F  T**  L  K  D  N  T  R  A  F  **N  V  S**  I  E  T  P  V

1201  CTTAGTGTTAATGCAACTTCGGTTCGACTTCAATCATGGCAAAGTGAAATGCTCAGAATGAATCCAAGAACTTCA
 401  L  S  V  **N  A  T**  S  V  R  L  Q  S  W  Q  S  E  M  L  R  M  N  P  R  T  S

1276  TTTGCAAAACGTTTTGCCAAATGGGCTCTACCCCTCTACTATAAACCAACGTGTGTTGTGGATTCTTCCTACATC
 426  F  A  K  R  F  A  K  W  A  L  P  L  Y  Y  K  P  T  C  V  V  D  S  S  Y  I

1351  AATACATTCGACAATTTTACATACAGTGCTCATCATATTGTACAGAACGATGCTTTCTACACTATATTGGATATT
 451  N  T  F  D  **N  F  T**  Y  S  A  H  H  I  V  Q  N  D  A  F  Y  T  I  L  D  I

1426  CCTCAAAAGTTTAATATGGAATACTTTAAAGTTGCATTCAAACCAACATCCCCAGTTCCAAATATGCAGAGGGAA
 476  P  Q  K  F  N  M  E  Y  F  K  V  A  F  K  P  T  S  P  V  P  N  M  Q  R  E

1501  GTCTTAGTGTTCCTGAGAAATGCAAAAATAGAATTGAAACCTAATCAAGGAATGCCAGAAGTTTACGTAGAAGGT
 501  V  L  V  F  L  R  N  A  K  I  E  L  K  P  N  Q  G  M  P  E  V  Y  V  E  G
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Figure 2.

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1576  AAAAGAGTGGATTATAACCACCACCATTCAACTGACTTGAATGTGAGTCAAGACCGGATTGGTTATGTCTATGCC
 526  K   R   V   D   Y   N   H   H   H   S   T   D   L   N   V   S   Q   D   R   I   G   Y   V   Y   A

1651  CTTCCAACAAAGGCTGCTCATATTGTATTTCCTTCATACGAGATCGAAATGTTTTACGATGGCTCCAGGATTATG
 551  L   P   T   K   A   A   H   I   V   F   P   S   Y   E   I   E   M   F   Y   D   G   S   R   I   M

1726  ATACAAGCTTCAAATATGTACCGAAATTTCACCAAAGGTCTCTGTGGTAACATGGATGGAGAATTCGTGAACGAC
 576  I   Q   A   S   N   M   Y   R   N   F   T   K   G   L   C   G   N   M   D   G   E   F   V   N   D

1801  GTGTTGACTCCATGGGGCTGCTACGCCAAAGACATGGCACTCTTTGTAGCATCTTATGCCGACAACAGCAACAGC
 601  V   L   T   P   W   G   C   Y   A   K   D   M   A   L   F   V   A   S   Y   A   D   N   S   N   S

1876  GAAGTAAGGAAGATCAAGGCTACCCAAAACGAGCAGACATGCGTCCCACAGTTTCATCAACCTCTAGTCAGCCAT
 626  E   V   R   K   I   K   A   T   Q   N   E   Q   T   C   V   P   Q   F   H   Q   P   L   V   S   H

1951  CAAATGAGATTGTCTCAAGTCATTAAGTTAGCAGATACCTCTTCCTCCTCTGAATCTTCCTCTTCATCAGAATCT
 651  Q   M   R   L   S   Q   V   I   K   L   A   D   T   S̲   S̲   S̲   S̲   E̲   S̲   S̲   S̲   S̲   E̲   S̲

2026  CATGAAAACAATTCATCACCATCTTCTGAGTCTCAAGTCAACAAGTCTAAGCGTCAACCAAATTCCAGACCAAGA
 676  H   E   N   N   S   S   P   S   S   E   S   Q   V   N   K   S   K   R   Q   P   N   S   R   P   R

2101  TCCAGTTCCAGTTCCAGCTCCAGCTCCAGTTCTGAATCCAATGAATCTGTCCTTGCCAAGAAAATCATTAACAAC
 701  S̲   S̲   S̲   S̲   S̲   S̲   S̲   S̲   S̲   S̲   E̲   S̲   N   E   S   V   L   A   K   K   I   I   N   N

2176  CAAATTGGACCAAAGCCTACTCTAATACCATCACAGTCTCCTATGACGTCCGATGACAAATGCATGACACAACAA
 726  Q   I   G   P   K   P   T   L   I   P   S   Q   S   P   M   T   S   D   D   K   C   M   T   Q   Q

2251  CCAAGACACACTTATTATGAAAACCAATTCTGTGTCAGTGAAAAGCCCCTGGACACATGCATGCCCCTTATTTGC
 751  P   R   H   T   Y   Y   E   N   Q   F   C   V   S   E   K   P   L   D   T   C   M   P   L   I   C

2326  CATGCAACTGAATCCTATACAATAGATGTAAATTTTTACTGTGTTCCACTAGGTCCTGCAGCTAATCATTACATG
 776  H   A   T   E   S   Y   T   I   D   V   N   F   Y   C   V   P   L   G   P   A   A   N   H   Y   M

2401  AAACTTGTCAAGAAAGGCATTCTCCCAGATTTAAGTCGCAACAGGAATGGTAAGAGGGTAGTTCTTCCTGTTGAA
 801  K   L   V   K   K   G   I   L   P   D   L   S   R   N   R   N   G   K   R   V   V   L   P   V   E

2476  ATCCCAATACAATGCGAACCTGTTTTAAACTGAAATCCGATTCTTGTTAATGGACTTAATTTAAAAAATCTGACT
 826  I   P   I   Q   C   E   P   V   L   N   *

2551  TATGAAGAAACTGGAATATTTTCAAAAATTGAACTATTTATGTATTT*AATAAATAAA*GAATGATAGCAACTTGA
2626  AAAAAAAAAAAAAAAAAAAA
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Fig. 2.   Partial nucleotide sequence and deduced amino acid sequence of vitellogenin cDNA of *Blattella germanica* (database accession number: AJ005115). Boldface letters, putative asparagine-linked glycosylation sites; boldface italic letters, polyadenylation signal; double underlines, clusters of serine residues; single underline, putative cleavage sites; asterisk, stop codon.

tively high level of conservation in certain motifs typical of vitellogenin sequences, including that of *B. germanica*.

Expression of the Vitellogenin Gene

Northern blot analyses were carried out using the fluorescein-labeled vitellogenin cDNA insert from the three clones as respective probes. The analyses showed here (Figs. 4, 5) correspond to the 1.7-kb insert from the Vg16a clone, although those of Vg3b and Vg8a gave the same results. To study the tissue and sex specificity of the vitellogenin gene expression, we used RNA from fat body samples from female last-instar nymphs, adult males, and adult females, and from the ovaries of adult females. The vitellogenin RNA had a length of ca. 6.2 kb (cf. RNA molecular-weight markers not shown), which means that the portion sequenced (2.6 kb) represents approximately one-third of the entire vitellogenin RNA. The transcript was only detectable in RNA from fat body of 2-day-old and 4-day-old adult females,

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AEDES1276-  1276  MSSWPYTGYKDITDMRPIAENPNAKIVHDDNQSTKTMEHTFGQDMTGVALRFHAKYDFDLINFQQFWSLIQKNDFV
BLATTELLA      1  FGTSPYTTIHNVPDFETVYMDQLFKLIH..VRKTAHFEKKMGEN.TGIVFKVKYDTDQEFLDTKWFLDEFKVLQLF

AEDES1276-  1352  SAVNYPFAYQPYEYHQFNLFYDSQRT..HAKSF.......KFY......AYQKFGAPSFEETGPKHPANRHSYSGN
BLATTELLA     74  TGLNYDVPTKDIFYNNLTVVYYDHEDTKNHAVSFTVTKEQSKFYETLNPVVQQNLKLSSGKKQKHRNVKSHRIRREY

AEDES1276-  1413  YYESNYAQPFVYSPGSQRRYEQFFRNAASGIRNSFVRYYDFGFEFYAPQYKSEFTFTTAFADSPVDKTSRQLYYFY
BLATTELLA    150  TEDENPAIPKDKQPNSHPRRQEYLSKSMALTGDATAVVLDMTLKFEGPA.ESYFTTTVSHATSLVNGSSNYL....

AEDES1276-  1489  ASPMFPSQSYFKDIPFSGKQFQFCATATSEFPRVPYLKFSDFDKYYGDASQYFDFLYGESCQGGAHIAVKGKQKQT
BLATTELLA    221  ...LFYDQHYYEE....KKRNQFCLSWSVYKPQVPIMNIYSAFEFDPNSKVHAIMNIGKECENGGSAVANIDMLRL

AEDES1276-  1565  GKYREYLRFSDVAKACKEQM.ANGYYQFEECQQAIDQAYYYDFYDYAIEYK.DVGSVAKNLTNKFYNYFQYAFYPY
BLATTELLA    290  SEHLDYVKNLTVSKLCDHEMRTKRDHVLPACRNSTERASDLNRVHVDINYNLKQHETFKRRVYKVYDFVRTHLYPH

AEDES1276-  1639  FESNFFYHGKSNYIKAEFEFAPYGDYYNASFFGPSYAFQVQNYPVFNDYSTYFPYFFKYTFFPRYQPYYMHRLPSH
BLATTELLA    366  VSEDVIVDNPAQFISANFTLKDNTRAFNVSIETPVLSVNATSVRLQSWQSEMLRMNPRTSFAKRFAKW...ALPL.

AEDES1276-  1715  KPRNRPYYELSNYEQFAIFDRKPQYPSCSFSNDYFYTFDNKKYFYDMGECWHAVMYTVKPDYDFYAQQSHFYN.SD
BLATTELLA    438  ......YYK.................PTCVVDSSYINTFDNFTY.....SAHHIV............QNDAFYTILD

AEDES1276-  1790  FEYKYKNGFEEYEQFAALARRGSDNQLYFKFLFGDN.YIEVFPNNGGVPFVKYNGRPYDISKSNIAHFEYKEGYPS
BLATTELLA    475  IPQKFN...MEYFKVAFKPTSPVPNMQREVLVFLRNAKIELKPNQ.GMPEVYVEGKRVDYNHHHSTDLNVSQD...

AEDES1276-  1865  FPFFYAFAYPNKDLEVSFFGGKLKFATDGYRARFFSDYSFYNNFVGLCGTNNGEYFDEFVTPDQCYMRKPEFFAAS
BLATTELLA    544  .RIGYVYALPTKAAHIVFPSYEIEMFYDGSRIMIQASNMYRNFTKGLCGNMDGEFVNDVLTPWGCYAKDMALFVAS

AEDES1276-  1941  YAITGQNCTGPAKAFNYAYQQKAKQECVKREVYYGDIIYNQEYYHPRYRYYNHNVEESSSSSSSSSSDSSSSSSSS
BLATTELLA    619  YADNSNSEVRKIKA......TQNEQTCVPQ..FHQPLVSHQMRLSQVIKLADTSSSSESSSSSESHENNSSPSSES

AEDES1276-  2017  EF.......SSLSRSGSSSSSSSSSEEQKE..................FHPHKQEHSMKECPVQH.QHQFFEQGDRI
BLATTELLA    687  QVNKSKRQPNSRPRSSSSSSSSSSSESNESVLAKKIINNQIGPKPTLIPSQSPMTSDDKCMTQQPRHTYYE..NQF

AEDES1276-  2067  CFSLRPLPVCHSK.CAATEKISKYFDVHCFEKDSTQAKKYKSEIGRGYTPDFKSFAPHK..TYKFNYPKSCVYKAY
BLATTELLA    761  CVSEKPLDTCMPLICHATESYTIDVNFYCVPL.GPAANHYMKLVKKGILPDLSRNRNGKRVVLPVEIPIQCEPVLN
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Fig. 3. Alignment of deduced amino acid partial sequence of *Blattella germanica* vitellogenin described herein (database accession number: AJ005115) with the homologous fragment from the mosquito *Aedes aegypti* described by Chen et al. (1994) (database accession number: U02548).

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Lymantria        1410  PFCTVDGTKVKTFSNRDYNVTLPEIWTVLMHAQT....NWEELVVLAKRPNEA
Bombyx           1449  PYCSIDGTRIHTFSNRSYEYPLSRSWHVVMQDESTQRGNWHELAILSRRQQR.
Athalia          1543  PTCVIDNNQASTFDNKTYPIELGNCWHVVMTTIPDDEQ.........NGD...
Pimpla           1468  RICVLDSHSATTFDESTYPINLGKCWHVVMTTFPKTNK.........NSG...
Aedes            1740  PSCSFSNDYFYTFDNKKYFYDMGECWHAVMYTVKPDYDFYAQQSHFYNSDFEY
Blattella         441  PTCVVDSSYINTFDNFTY.....SAHHIVQNDAFYTILDIPQKFNMEYFKVAF
Anthonomus       1466  PTCVIDQTTAQTFSNKTYSVNLDKEPTVVMQYVPKDARVNGQQSKSVEQLLRE
Caenorhabditis   1295  ATCKVDQSEVSTFHNVIYRAPLTTCYSLV.....................

Lymantria        1459  KAKREIGKIGKLDLYISHKTATGKHLEVNIPYS....AANNKANVK.VETNAQ
Bombyx           1501  .........DQQEIYISYKSESGQDLEIEIQPA....SGDSAYQVK.VTTNTK
Athalia          1584  ...DEIPEDMRVSVVCRDINEHQRECRLVLGEAEIEMYPNNDQAA..IKVNGR
Pimpla           1509  .SAEPIDEDMALSILVRDAGDKKKDIKVTLGDKELQFSHADSKNK..VTLDGK
Aedes            1793  KYKNGFEEYEQFAALARRGSDNQLYFKFLFGDNYIEVFPNNGGVPF.VKYNGR
Blattella         489  KPTSPVP............NMQREVLVFLRNAKIELKPNQG.MPE.VYVEGK
Anthonomus       1519  SIENYVVLVRQVAANQKEVIINLNHPRTQGKTVKIEMKPSEDRQKS.ARNPAA
Caenorhabditis   1324  ..AKDCSEQPRFAVLAKKINKNSEELLVKVVRREEEIVVKKSDDKFLVKVDGK

Lymantria        1507  LVADGDLTTYWDDVAE....TPLLQYSNHPDRVLLLHLSDG.LHLLFDGKRGI
Bombyx           1540  KITDDDLTMYWDDVKE....QPFLQYHTHKDGVLVINIEDDRIRAIYDGQRFV
Athalia          1632  AVQLSQEETYRYTSQNG.EI..QFEAYLKPSGVVYIHSEEYGVRALYDGERIK
Pimpla           1559  KVDLSEKRSYRH..KNGKDI..DFEVLQRPDGTLGLVSSKYDIDAWYDGQRVQ
Aedes            1845  PYDISKSNIAHFEYKEGYPSFPFFYAFAYPNKDLEVSFFGGKLKFATDGYRAR
Blattella         527  RVDYN....HHHSTDLNVSQDRIGYVVALPTKAAHIVFPSYEIEMFYDGSRIM
Anthonomus       1571  KVTIDGQEMHFDDKQIADKCDGYVQVYALPNGEVKLEVED.AFYLIYDGQRVK
Caenorhabditis   1375  KVNPTELEQYNIEI...............LGDNLIVIRLPQGEVR..FDGYTVK

Lymantria        1555  FTTS..QYRNITRGICGQNSGDPLDDYKTPLG.IVDHSQHFGAAFTLDLEKT
Bombyx           1589  VFTQ..DYRNSTRGICGRMSGEQRDDYLTPEG.LVDKPELYAAAYSLNEENS
Athalia          1682  LILN.NEYRDQIRGLCGTFNGEPATDFTAPQNCILKNPEHFAASYALTKDQC
Pimpla           1608  IKAS.GKYRSDIRGLCGNFDGEPDNDFTSPKDCVLLKPEEFAASYALTTKDC
Aedes            1898  FFSD.YSFYNNFVGLCGTNNGEYFDEFVTPDQCYMRKPEFFAASYAITGQNC
Blattella         576  IQAS.NMYRNFTKGLCGNMDGEFVNDVLTPWGCYAKDMALFVASYADNSNSE
Anthonomus       1623  VTATGNKLRDSVYGLCGRFSQDKHEDFTVPSNCVTRDTRKFVESYQVEKGQQ
Caenorhabditis   1412  TNMPSVASQNQLCGLCGNNDGERDNEFMTADNYETEDVEEFHRSYLLKNEEC
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Fig. 4. Alignments of the subdomain IV (Chen et al., 1997) of the vitellogenin sequences of 7 species of insects: boll weevil, *Anthonomus grandis* (Trewitt et al., 1992; M72980); yellow fever mosquito, *Aedes aegypti* (Chen et al., 1994; U02548); silkworm moth, *Bombyx mori* (Yano et al., 1994a; D13160); gypsy moth, *Lymantria dispar* (Hiremath and Lehtoma, 1997a; V60186); turnip sawfly, *Athalia rosae* (Nose et al., 1997; AB007850), the parasitoid wasp *Pimpla nipponica* (Nose et al., 1997; AF026789); German cockroach, *Blattella germanica* (present study; AJ005115); and the nematode *Caenorhabditis elegans* (vit-5: Spieth et al., 1985; X03044). The PILEUP program from the GCG package was used, and results were displayed by BOX, from the same package.

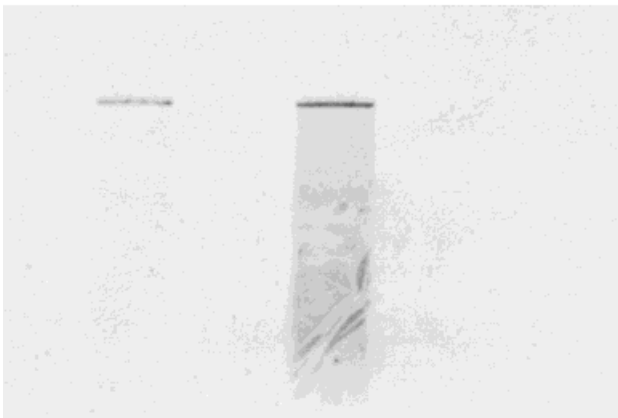
6lFB 2fFB 2fOV 4fFB 4fOV 4mFB

Figure 5.

whereas it was not detected in samples from ovary tissues or from fat body tissues from adult males or female nymphs (Fig. 5), even after extended autoradiographic exposures of the blots (not shown).

The same hybridization techniques were used to study the stage specificity of the vitellogenin gene expression in the fat body of adult females during the first vitellogenic cycle. The

Fig. 5. Northern blot analysis of total RNA (5 μg each) from different tissues of *Blattella germanica*: fat body from 6-day-old last-instar female nymph (6l FB); fat body from 2-day-old adult female (2f FB); ovary from 2-day-old adult female (2f OV), fat body from 4-day-old adult female (4f FB); ovary from 4-day-old adult female (4f OV); fat body from 4-day-old adult male (4m FB). The insert of Vg16a was used as probe.

results (Fig. 6) showed that mRNA was first detectable at day 1 of adult life, reaching maximal levels on day 5, and then decreasing on days 6 and 7. The pattern of temporal changes in mRNA levels during the first vitellogenic cycle is approximately parallel to that of vitellogenin protein production in the fat body as described by Martín et al. (1995a,b), although vitellogenin content in the fat body seems to decline before the mRNA levels reach a maximum (Fig. 6).

DISCUSSION

A partial cDNA clone for the vitellogenin gene from the cockroach *B. germanica* has been isolated from a cDNA expression library using an anti-vitellin–vitellogenin antiserum (Martín et al., 1995a) probe. The cDNA inserts of three positive clones were studied; nucleotide sequence analyses showed that they all contained fragments of
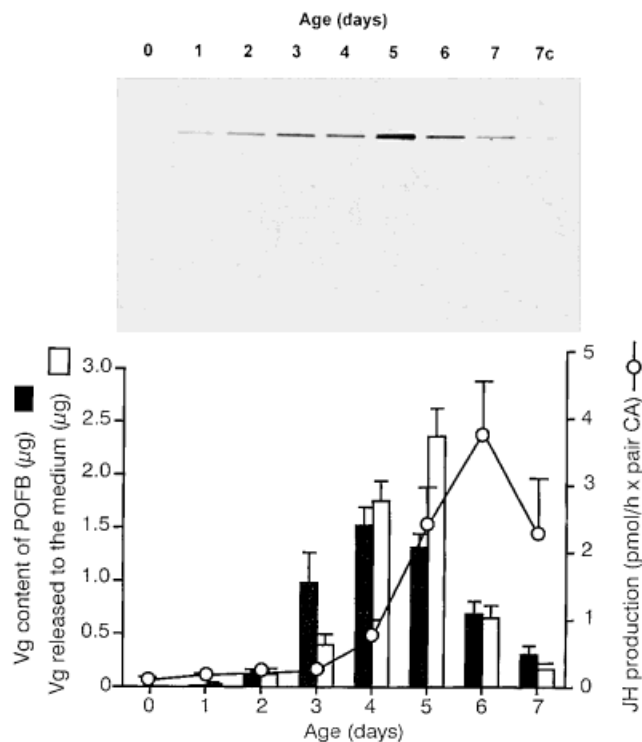

Fig. 6.   Northern blot analysis of total RNA (5 µg each) from fat body from females of *Blattella germanica* during the first 7 days of adult life. 7c, 7-day-old adult female with the basal oocytes chorionated. The insert of Vg16a was used as probe. For comparative purposes, the data for vitellogenin (Vg) protein produced in vitro by periovaric fat body (POFB) (from Martín et al., 1995b), and juvenile hormone synthesis in vitro by corpora allata (from Maestro et al., 1994) during the first vitellogenic cycle of *B. germanica* are shown. Results in the graphs are expressed as the mean ±SEM (n = 7–14).

the same vitellogenin sequence. The longest cDNA sequence obtained was 2,645 nucleotides long and represents the 3′ region of the *B. germanica* vitellogenin cDNA, including the stop codon, the polyadenylation signal and the poly(A) tail. The deduced amino sequence is 825 residues long and is similar to the homologous portion of the vitellogenin of other insect species, especially that of the mosquito *A. aegypti*.

The amino acid sequence of *B. germanica* includes polyserine clusters towards the 3′ end, which are also present in the homologous sequence of the mosquito species. Moreover, 13 putative glycosylation sites, and 2 putative cleavage sites were identified. These putative cleavage sites are not surrounded by serine rich domains, which is also the case in *L. dispar* (Hiremath and Lethoma, 1997a). Cleavage in these sites would give polypeptides corresponding to the small subunits (50–95 kDa) described in mature vitellin (Wojchowski et al., 1986) (see also Purcell et al., 1988; Martín et al., 1995a) and also in the hemolymph of ovariectomized females of *B. germanica* (Martín et al., 1996).

Alignment and comparison of the subdomain IV of vitellogenin (Chen et al., 1997) in all insect species studied thus far and in the nematode *C. elegans* show a high degree of conservation; the coincidence of the motif GL/ICG is particularly interesting. The same, although slightly longer, motif (TCGLCG), was observed by Mouchel et al. (1996) to be common in various vertebrate vitellogenins (*Xenopus laevis*, *Gallus gallus*, *Acipenser transmontanus*, *Fundulus heteroclitus*, *Ichtyomyzon unicuspus*, and *Oncorhynchus mykiss*), and in human von Willebrand factor in domains D_1 and D_2 and the human intestinal mucine 2 glycoprotein in domain D_3. The same investigators also noted the coincidence with the CGLCG motif of *C. elegans*, and with the GLCG motifs of *A. aegypti* and *B. mori*.

Northern blot analysis indicated that vitellogenin gene expression is limited to the fat body of adult females, as expected. The pattern of expression during the first vitellogenic cycle is similar to that of vitellogenin production by the fat body, studied both in vivo and in vitro by Martín et al. (1995a,b), although vitellogenin protein in the fat body appears to peak on day 4 whereas vitellogenin mRNA peaks on day 5 (Fig. 5). This suggests the hypothesis of an inhibitory mechanism on vitellogenin translation occurring on day 5.

Our previous observations concerning the relationships between juvenile hormone and vi-

tellogenin indicated that the juvenile hormone cycle (Bellés et al., 1987; Maestro et al., 1994) is not parallel to that of vitellogenin protein (Martín et al., 1995a,b). In particular, it is quite apparent that vitellogenin production begin to decline from day 5, whereas rates of juvenile hormone synthesis by the corpora allata are still increasing at this stage (Fig. 6). The present results reinforce such a divergence by showing that the accumulation of vitellogenin mRNA increases with increased juvenile hormone synthesis, but declines before the production of this hormone reaches a maximum (Fig. 5). This suggests the hypothesis that increased titers of juvenile hormone could be involved in a feedback mechanism terminating vitellogenesis, perhaps causing a direct or indirect suppression of the vitellogenin mRNA.

In any case, the studies reported in this paper show that the isolated cDNA seems to be specific for the vitellogenin mRNA of *B. germanica*, and that it can be used as a tool to study the molecular action of potential regulators of vitellogenesis, and to test new control hypothesis, such as those just proposed.

ACKNOWLEDGMENTS

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