Construction and partial characterization of a recombinant DNA probe for locust vitellogenin messenger RNA

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Double-stranded DNA complementary to poly(A)-containing RNA from the fat body of adult female locusts, *Locusta migratoria*, was synthesized. Hybrid molecules containing this cDNA was constructed in the *Pst*I site of the plasmid pAT 153 by the technique of dC·dG tailing and amplified in *Escherichia coli* K-12 strain HB 101. Ten colonies of bacteria were identified as carrying recombinant plasmids containing DNA complementary to locust vitellogenin mRNA by (a) 'Northern' blot hybridization analysis and (b) hybrid selection of vitellogenin mRNA and immunological detection of the products of translation of the mRNA. Of the ten recombinant plasmids, one, termed plasmid 4E, containing a cDNA insert of about 650 nucleotides, was characterized in greater detail and a partial restriction map obtained. Using this hybrid plasmid it was possible to derive a value for the average content of vitellogenin mRNA in the adult female locust fat body as 1.5×10^5 molecules/cell, and to establish that the haploid genome of *L. migratoria* contains only one or two genes coding for vitellogenin.

During oogenesis in oviparous vertebrates and in most invertebrates, the synthesis of several egg proteins is under hormonal control (Tata & Smith, 1979; Hagedorn & Kunkel, 1979; Wahli *et al.*, 1981). Under the influence of juvenile hormone the fat body cells of many adult female insects produce large amounts of a high-molecular-weight (200–250 kDa) yolk protein precursor, vitellogenin, which after post-translational modifications, appears in the haemolymph as aggregates of components ranging in size from 180 to 45 kDa (Wyatt, 1980; Engelmann, 1980). These are then taken up by the developing oocyte to form yolk, also termed as vitellin (Chen, 1980). In the higher Diptera, as exemplified by *Calliphora erythrocephala* and *Drosophila melanogaster* (Riddell *et al.*, 1981), a similar vitellogenin-like precursor is not found but only vitellin, which is entirely composed of polypeptides of about 50 kDa.

Our laboratory has been studying the evolutionary aspects of vitellogenin genes in vertebrates and invertebrates (Tata & Smith, 1979; Applebaum *et al.*, 1981), for which we have chosen vitellogenesis in the locust fat body as a model for the insect system. In

order to extend our previous studies on locust mRNA (Applebaum *et al.*, 1981) to the structure and organization of locust and other insect vitellogenin genes, it became necessary to clone in plasmids DNA complementary to the locust vitellogenin mRNA to be used as a probe for screening genomic DNA clone libraries from various insects. In this paper we describe the preparation of cloned cDNA for locust vitellogenin mRNA and its partial characterization. Using this probe we have quantified vitellogenin mRNA sequences in the fat body of vitellogenic female locusts, and determined the copy number for locust vitellogenin genes by two different methods.

Materials and methods

Materials

Organisms. Vitellogenic female locusts (*Locusta migratoria*) were a gift from The Centre for Overseas Pest Research, London. *E. coli* K-12 strain HB 101 and pAT 153 plasmid were a gift from Dr. R. A. Flavell, National Institute for Medical Research, London.

DNA, enzymes and other materials. Plasmid pAT 153 DNA, used as the vector to transform *E. coli* HB 101, was purified according to Clewell & Helinski (1969). Avian myeloblastosis virus reverse transcriptase was provided by Dr. J. W. Beard (Life

Abbreviations used: cDNA, complementary DNA; poly(A), polyadenylic acid; SDS, sodium dodecyl sulphate.

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Sciences Inc., St. Petersburg, FL, U.S.A.). Terminal transferase and poly(A) were obtained from PL Biochemicals, Milwaukee, WI, U.S.A. DNA polymerase I (EC 2.7.7.7) and restriction endonucleases, other than *Pst*I, were purchased from Bethesda Research Laboratories, Gaithersburg, MD, U.S.A. Restriction enzyme *Pst*I was from New England Biolabs, Beverly, MA, U.S.A. S_1 nuclease (EC 3.1.30.1) and fixed *Staphylococcus aureus* cells were purchased from Miles, Slough, U.K. Proteinase K was obtained from Boehringer. Nitrocellulose membrane filters were from Schleicher & Schüll (Dassel, Germany). All other enzymes and biochemical reagents were from Sigma and BDH.

Radioactive materials. [^3H]dCTP (specific radioactivity 20 Ci/mmol), [^3H]dGTP (11 Ci/mmol), [α - ^{32}P]dCTP and [α - ^{32}P]TTP (2500–3000 Ci/mmol) were obtained from Amersham International.

Construction of recombinant plasmids

Poly(A)-containing RNA enriched for locust vitellogenin mRNA was isolated from total locust fat body RNA and single-stranded cDNA was synthesized according to Applebaum *et al.* (1981). RNA was removed by alkali treatment with 0.2 M-NaOH for 20 min at 68°C, and the second DNA strand was synthesized with *E. coli* DNA polymerase I (Hobart *et al.*, 1980). For homopolymer tailing with dCTP, double-stranded cDNA was made blunt-ended with single-strand-specific S_1 nuclease (Goodman & MacDonald, 1979) and was tailed with [^{32}P]dCTP (10 Ci/mmol) using calf thymus terminal transferase (Nelson & Brutlag, 1979). *Pst*I-digested pAT 153 plasmid was tailed under identical conditions with [^3H]dGTP.

Reannealing and transformation

Equimolar quantities of the dC-tailed cDNA (1500 bases average length) and dG-tailed pAT 153 (1 µg/ml) were annealed and the resultant hybrid molecules were used to transform *E. coli* K-12 strain HB 101 according to Wensink *et al.* (1974).

Screening of recombinant plasmids

Twenty-two tetracycline-resistant clones were obtained from 12 ng of double-stranded cDNA. To screen for locust vitellogenin cDNA-containing sequences, recombinant colonies were grown in four batches of five individual colonies, plasmids isolated (Birnboim & Doly, 1979) and digested with *Eco*RI restriction enzyme. Locust poly(A)-containing RNA was resolved by electrophoresis on an agarose gel (McMaster & Carmichael, 1977) and the RNA was transferred to nitrocellulose filters (Thomas, 1980). The RNA on the filters was hybridized (Cleveland *et al.*, 1980) with nick-translated (Rigby *et al.*, 1977) group-isolated plasmid DNA. Groups which showed strong positive hybridization with full-size (31S)

vitellogenin mRNA from female locust poly(A)-containing RNA were grown individually and the experiment was repeated with individual plasmids.

Hybrid selection and translation

Plasmid DNA was immobilized on diazotized aminothiophenol paper (Reiser & Wardale, 1981), and 100 µg of total or 5 µg of poly(A)-containing locust fat body mRNA was hybridized to the immobilized plasmid DNA and the hybridized RNA was eluted and reprecipitated as described by Smith *et al.* (1979). Hybrid-selected RNA was translated in a rabbit reticulocyte lysate cell-free translation system (Applebaum *et al.*, 1981) and the products were separated on an SDS/9% polyacrylamide gel (Laemmli, 1970). Translation products were also immunoprecipitated with locust antivitellogenin antibody (Harry *et al.*, 1979) and staphylococcal protein A (Bollen *et al.*, 1981) and the immunoprecipitates were electrophoresed as above.

Restriction enzyme analysis of cloned cDNA

The conditions for restriction endonuclease digestions were those recommended by the supplier. The cDNA insert was eluted after electrophoresis in a 1.8% agarose gel according to McDonnell *et al.* (1977), purified by DE-52 cellulose chromatography (Smith & Birnstiel, 1976) and used for restriction analysis. The plasmid, with or without the insert, was digested with *Pst*I alone or with one other restriction endonuclease (Robinson & Ingram, 1981).

Determination of gene number

Two different methods were used, namely solution hybridization analysis based on acceleration of probe annealing (Sharp *et al.*, 1974) as described by Kurtz (1981) and saturation hybridization analysis (Sala-Trepat *et al.*, 1979). Sheared *E. coli* DNA was used as a control to monitor the self-annealing in the presence of various amounts of the probe.

Isolation of high molecular weight DNA and restriction analysis

Fat bodies from female or gonads from male locusts were homogenized under very mild conditions in lysis buffer [10 mM-Tris/HCl (pH 7.5)/0.35 M-NaCl/1 mM-disodium EDTA/7 M-urea/2% SDS/0.5% 2-mercaptoethanol] and the DNA was isolated as described by Keene *et al.* (1981) except that the nucleic acids were precipitated with 2 vol. of cold ethanol and the floating high molecular weight DNA was recovered and dissolved in 10 mM-Tris/HCl (pH 8.0)/1 mM-EDTA/200 mM-NaCl and treated with ribonuclease A (40 µg/ml) for 1 h at 37°C.

DNA was restriction digested, as recommended by the suppliers of restriction enzymes, and the digested DNA was electrophoretically separated on a 0.75% agarose gel and transferred to nitrocellulose paper (Southern, 1975), as modified by Wahl *et al.* (1979). The DNA on filters was hybridized with ^{32}P -labelled plasmid 4E as described for filter-bound RNA.

Isolation of total RNA from individual fat bodies

Total nucleic acids were isolated from vitellogenic female locust fat bodies (each weighing 70–100 mg) as described for the isolation of high molecular weight DNA, except that the precipitate was collected by centrifugation at full speed for 5 min in a Beckman Microfuge. The dried pellet was taken up in 100 μl of 50 mM-Tris/HCl (pH 7.6)/5 mM-MgCl₂/10 mM-2-mercaptoethanol, 20 μg of ribonuclease-free deoxyribonuclease/ml (Zimmerman & Sandeen, 1966) was added and the mixture was incubated at 37°C for 1 h. The solution was then treated with proteinase K, extracted with phenol, and the RNA in the aqueous phase was precipitated with 2 vol. of redistilled ethanol. The pellet was collected and washed twice with 100 μl of ice-cold 3 M-sodium acetate, pH 5.5, and finally washed twice with ethanol/water (7:3, v/v), once with ethanol and dried *in vacuo*. The pellet of RNA was dissolved in 15–20 μl of sterile distilled water and kept frozen at –70°C until further use.

Results and discussion

Construction of hybrid plasmids

Our previous studies on locust fat body vitellogenin mRNA had shown that complementary DNA synthesized from poly(A)-containing mRNA ranged in size from 1000 to 3500 nucleotides (Applebaum *et al.*, 1981). We decided to use this single-stranded cDNA preparation for second strand synthesis without any size selection for high molecular weight components, since one of our goals was eventually to screen the recombinant plasmids for DNA sequences complementary to other messengers besides vitellogenin mRNA. The procedures used for double stranding and homopolymer tailing were first standardized in this laboratory for cloning *Xenopus* oviduct poly(A)-containing RNA sequences (T. C. James & C. A. Maack, unpublished work). Under these conditions 65–70% of the single-stranded cDNA was rendered double-stranded. Homopolymer tailing with dCTP of double-stranded cDNA and with dGTP of the plasmid pAT 153 (Twigg & Sherrat, 1980) using deoxynucleotidyl transferase resulted in about 50–100 nucleotides being transferred to the 3' ends of the respective substrates. Hybrid plasmids were then used to transform an *E. coli* HB 101 rec A[–] strain with a trans-

formation frequency of 10⁶ colonies/ μg for pAT 153, 0.5 $\times$ 10³ colonies/ μg for the hybrid plasmid and no colonies with only the tailed plasmid.

Identification and partial characterization of recombinant plasmids containing locust vitellogenin cDNA sequences

Twenty-two tetracycline-resistant colonies were grouped into four batches and the plasmids were isolated. All four batches of nick-translated plasmids hybridized to a 31S RNA from locust fat body poly(A)-containing RNA transferred to nitrocellulose filters (Fig. 1a, sets A–D). 31S is the size expected for locust vitellogenin mRNA (Applebaum *et al.*, 1981). Two other RNA species of low molecular weight also hybridized to one pooled plasmid preparation. When each colony was grown individually, 10 recombinant plasmids were found to hybridize to locust vitellogenin mRNA by the same procedure (results not shown). The size of the cDNA inserts ranged from 400 to 650 nucleotides, that of plasmid 4E being the maximum (Fig. 1a, set E). Many restriction enzymes were found to cleave the inserted DNA of plasmid 4E at several sites (Fig. 1b). *Msp*I, *Alu*I, *Hae*III and *Taq*I cut more frequently than did *Hae*II or *Tha*I. It is possible that the sites for *Hae*III and *Tha*I near the two ends of the insert were artificially generated as a result of the procedures of homopolymer tailing and annealing of the plasmid.

Hybrid selection of vitellogenin mRNA and its translation

In order to ascertain the presence of vitellogenin mRNA sequences in the recombinant plasmid, fat body RNA was hybridized to plasmid 4E DNA, eluted, and the putative vitellogenin mRNA was isolated and translated in a rabbit reticulocyte lysate system. When the resultant [³⁵S]methionine-labelled products were immunoprecipitated (Bollen *et al.*, 1981) with locust antivitelin antibody (Harry *et al.*, 1979), the translation products contained a high molecular weight protein of 150 kDa and a low molecular weight protein of approx. 45 kDa, with four faintly discernible bands of proteins of approx. 190 kDa (Fig. 2). This result led us to conclude that plasmid 4E contains locust vitellogenin coding sequences. However, no full-length (220 kDa) vitellogenin was detected after immunoprecipitation, although there were peptides of about 220 kDa present in the translation products before immunoprecipitation (results not shown). A similar result was obtained earlier with translation *in vitro* of locust vitellogenin mRNA in reticulocyte lysate (Applebaum *et al.*, 1981), whereas other studies from our laboratory (C. D. Lane, J. Champion, A. Colman, T. C. James & S. W. Applebaum, unpublished work) indicate that locust vitellogenin

Fig. 1. 'Northern' blot analysis of recombinant plasmids containing inserted cDNA to locust fat body RNA and partial restriction map of plasmid 4E

(a) Plasmid DNA from 40 ml cultures was isolated, nick-translated, and hybridized to locust RNA electrophoretically separated on a 1% agarose gel and transferred to nitrocellulose filters as described in the Materials and methods section. Hybridization was visualized by autoradiography using preflashed X-ray film (Laskey, 1980). Sets A–D represent hybridization of DNA from plasmids pooled from twenty colonies grown as four groups of five each. Of the 20 plasmids analysed in such groups, ten gave positive hybridization signals at the position of locust vitellogenin mRNA when analysed individually. Set E represents hybridization of a single plasmid (plasmid 4E) which contained the longest cDNA insert. All groups contained plasmids hybridizing to authentic vitellogenin mRNA, while group B also contained plasmids hybridizing to other RNAs, indicated by the arrows. The RNA samples tested were: lane 1, 20 μ g of total RNA from female locust fat bodies; lane 2, 1 μ g of poly(A)-containing RNA from female locust fat bodies; lane 3, 20 μ g of total RNA from male locust fat bodies. (b) Plasmid 4E DNA was cleaved with different restriction enzymes and the sizes of the resulting DNA fragments were determined after electrophoresis on 6% acrylamide/bisacrylamide (20:1, w/w) gels stained with ethidium bromide. The restriction map is aligned with reference to the *Pst*I site in the parent plasmid pAT 153. Restriction enzymes: ▼, *Hae*II; ★, *Tha*I; ●, *Msp*I; ▲, *Hae*III; ■, *Alu*I; ◆, *Taq*I.

is correctly synthesized and processed in *Xenopus* oocytes injected with the same mRNA preparations. These results corroborate those in locusts (Harry *et al.*, 1979) demonstrating that locust vitellogenins are highly susceptible to proteolysis. The antibody preparations used in these immunoprecipitation reactions were adsorbed on male locust haemolymph so that it is possible that they contain a proteinase activity which could not be inhibited with our immunoprecipitation procedure.

Vitellogenin mRNA content in individual fat bodies

In order to determine the vitellogenin mRNA

content per fat body, total RNA was isolated from individual vitellogenic female fat bodies, covalently fixed to aminothiophenol-paper and hybridized to nick-translated plasmid 4E, in the same way as the procedure described for determining vitellogenin mRNA concentration in *Xenopus* liver (Searle & Tata, 1981). Using a 50% pure locust vitellogenin mRNA to derive a standard curve and recovering 135–150 μ g of total RNA per fat body in four experiments on individual fat bodies of 75–95 mg, we obtained a value of 600–700 ng of vitellogenin mRNA per fat body or about 0.5% of the total RNA. Assuming a 25% loss of RNA during extrac-

Fig. 2. SDS/polyacrylamide gel electrophoresis of [^{35}S]-methionine-labelled polypeptide products of translation of hybrid-selected RNA from vitellogenic female locust fat body in reticulocyte cell-free translation system

The hybrid-selected RNA was isolated as described in the Materials and methods section, translated in a micrococcal nuclease-treated rabbit reticulocyte lysate (Pelham & Jackson, 1976) and the translation products were immunoprecipitated (Bollen *et al.*, 1981). The immunoprecipitates were analysed by electrophoresis on an SDS/9% polyacrylamide gel, followed by fluorography (Bonner & Laskey, 1974). Lane 1, no RNA added; lane 2, RNA selected with plasmid pAT 153 alone; lane 3, RNA selected with plasmid 4E. Arrows indicate the major cleavage products of locust vitellogenin of 150 (top) and 45 (bottom) kDa.

tion (as judged from co-extraction of known amounts of carrier RNA), the number of cells per fat body as 1.4×10^6 (Chinzei *et al.*, 1982) and the known complexity of vitellogenin mRNA of 7100 nucleotides (Applebaum *et al.*, 1981), the vitellogenin mRNA content of fully vitellogenic fat body can be calculated as 1.5×10^5 copies per cell (Table 1). It would be most useful to carry out similar measurements at different developmental stages in order to determine the stage at which the vitellogenin gene is first activated. At the same time, the cloned cDNA probe would enable one to study in better detail how juvenile hormone regulates the transcription of this gene in the locust as well as in other insects (Engelmann, 1980; Wyatt, 1980).

Copy number of locust vitellogenin genes

High molecular weight DNA prepared from the female locust fat bodies or male locust gonads was cleaved with *EcoRI* or *BamHI* restriction endonucleases, electrophoretically fractionated on a 0.75% agarose gel and transferred on to nitrocellulose filters. It can be seen in Fig. 3(a) that *EcoRI* digestion yields two major bands of DNA of approx. 16000–17000 and 4500–5000 bases and a few less intensive bands of low molecular weight. Similarly, *BamHI* digestion also gave rise to two major DNA bands of 3000 and 4000 bases, although the position of the band around the 4000 base position is found to be different for the two different populations of locusts. The limited number of bands may be due to the short length of plasmid 4E used as the probe, or may indicate that locust vitellogenin is indeed encoded by a single copy gene.

In order to obtain a more accurate value for the number of vitellogenin genes in the locust genome, the cDNA insert was excised from plasmid 4E, nick-translated and used for solution hybridization according to two different procedures. In the first type of analysis, namely the accelerated probe annealing method of Sharp *et al.* (1974), the slope of the line shown in Fig. 3(b) for the annealing of the probe to locust genomic DNA is $n + 1$, where n is the copy number per haploid genome. This gave a value of $n = 1.5$, thus indicating only one or two locust vitellogenin genes per haploid genome. A reliable measurement of the number of genes coding for a particular protein can also be obtained by a saturation hybridization experiment in which fixed amounts of genomic DNA (400–500 base pairs) are annealed in the presence of a cDNA probe containing the sequences of the gene in question. In the second type of analysis, when such an experiment with sheared locust genomic DNA (50 μg) and varying amounts of nick-translated 4E insert was carried out, it showed that at saturation 8pg of the probe hybridized to 50 μg of genomic DNA (Fig. 3c). Therefore, locust DNA sequences homologous to

Fig. 3. Detection and determination of copy number of vitellogenin genes in the locust

the vitellogenin gene represented in the cDNA insert comprise a fraction of 1.6×10^{-7} of the locust genome. Since the haploid complement of DNA

(a) Detection of vitellogenin genes in locust genomic DNA. Locust genomic DNA (50 µg) prepared from animals obtained either from the Centre for Overseas Pest Research (A and B) or from the Hebrew University of Jerusalem (C and D) was digested to completion with restriction enzymes *Bam*HI (A and C) or *Eco*RI (B and D). The DNA fragments were electrophoresed in a 0.75% agarose gel, blotted on to nitrocellulose filters, hybridized to plasmid 4E DNA labelled with α - 32 P-labelled deoxyribonucleoside triphosphates by nick-translation (specific radioactivity 10^8 c.p.m./µg) and autoradiographed. (b) Acceleration of annealing of 32 P-labelled double-stranded locust vitellogenin cDNA in the presence of locust genomic DNA (■) or calf thymus DNA (▲). Annealing reaction mixtures contained 2 ng of labelled locust vitellogenin cDNA insert excised from plasmid 4E and 2 mg of locust genomic DNA or calf thymus DNA. Hybridization was carried out at 68°C in a total volume of 5 µl containing 1.0 M-NaCl, 0.14 M-sodium phosphate buffer, pH 6.8, and 0.1% SDS (Sharp *et al.*, 1974). Genomic DNA was degraded to approx. 400 nucleotides by boiling in 0.3 M-NaOH for 20 min. The mean size of the nick-translated cDNA probe was 400 nucleotides. The reaction mixture was sealed in siliconized capillary tubes, boiled for 10 min and incubated at 68°C. Capillaries were removed at various times and S_1 nuclease resistance was measured as described by Milcarek *et al.* (1974). The data were plotted as described by Kurtz (1981) where 1/fraction single-stranded represents the fraction of probe DNA which is single-stranded at time t and $t_{1/2}$ is the time required for half the probe to renature in the presence of 2 mg of calf thymus DNA. In this type of analysis, the slope of the line showing annealing to locust genomic DNA is $n + 1$, where n is the proportional gene copy number. The slope of the line shown for locust genomic DNA is 2.5, indicating 1–2 vitellogenin genes per haploid genome of locust. (c) Saturation analysis of the hybridization reaction of double-stranded locust vitellogenin cDNA with locust genomic DNA. Locust genomic DNA (50 µg) or *E. coli* DNA sheared to approximately 400 base pairs (Britten *et al.*, 1974) were mixed with increasing amounts of 32 P-labelled double-stranded locust vitellogenin cDNA (2×10^8 c.p.m./µg) insert excised from plasmid 4E in 5 µl of 0.3 M-NaCl/1 mM-EDTA/0.1% SDS/10 mM-Tris/HCl, pH 7.4. The reaction mixtures were sealed in siliconized capillary tubes, boiled for 10 min and incubated at 68°C for 72 h. At the end of the incubation period, duplex formation was measured by S_1 nuclease resistance (Milcarek *et al.*, 1974). Values for self-annealing measured in the presence of *E. coli* DNA were subtracted from experimental values, the radioactivity was corrected for decay and converted to pg of cDNA.

Table 1. *Vitellogenin mRNA content of individual locust fat bodies*

Weight of individual fat body (mg)	Total RNA per fat body* (μ g)	Vitellogenin mRNA per fat body (μ g)	Vitellogenin mRNA (% of total RNA)	$10^{-5} \times$ Number of mRNA sequences per cell
75	135 (180)	1.086	0.60	2.1
70	127 (170)	0.886	0.52	1.8
80	137 (182)	1.153	0.63	2.3
95	142.5 (189)	1.193	0.63	2.4

* Values in parenthesis are corrected for the recovery of RNA.

in locust is 6.35 pg (Rees *et al.*, 1978) or 6.35×10^9 base pairs, this fraction would be equivalent to approx. 1016 base pairs of locust vitellogenin DNA. As the size of the insert used in this analysis is 650 base pairs, this number indicates two vitellogenin genes per haploid locust genome. Thus, both types of solution hybridization analyses, as well as restriction endonuclease analysis of the genomic DNA, indicate that the vitellogenin gene in locust is a 'single copy' (1–2 copies) gene. The 'single copy' vitellogenin gene in locust contrasts with the multiplicity found in *Xenopus* (Wahli *et al.*, 1981) but is more analogous to that of the single copy gene in the chicken genome (Arnberg *et al.*, 1981). The fact that we have detected only one or two vitellogenin genes in DNA from fat bodies of fully adult, vitellogenic female locusts (Fig. 3) suggests that a tissue-specific amplification of this gene does not occur in the species of locust we have studied. This is in contrast to the 3–10-fold sex-specific amplification of egg chorion protein genes in *Drosophila* egg chamber cells at the time of oogenesis (Spradling & Mahowald, 1980) or that of genes coding for larval serum protein in female *Drosophila* fat body upon activation by the hormone ecdysone during metamorphosis (Roberts & Brock, 1981).

Our construction of the recombinant DNA plasmid will make it possible to obtain genomic DNA clones of vitellogenin genes of locusts and other insects. Meanwhile, we have already utilized the cloned 4E cDNA probe for a wider application in determining the conservation or divergence of vitellogenin coding sequences in different species of locusts, as well as among other insects and even in non-insect species (T. C. James, U. M. Bond, C. A. Maack, S. W. Applebaum & J. R. Tata, unpublished work). Although only a partial coding sequence has been cloned, DNA hybridization analysis with this vitellogenin cDNA probe revealed a high degree of coding sequence conservation between *L. migratoria* and another desert locust, *Schistocerca gregaria*. A longer sequence of cloned vitellogenin

cDNA will be necessary for extending such studies to the evolutionarily more distant insects and a full-length cDNA insert for studies on the structure of the locust vitellogenin mRNA and its gene.

This work was carried out under conditions of biological and physical containment at recommended by GMAG (Genetic Manipulation Advisory Group) and approved by the Biological Safety Committee of the National Institute for Medical Research.

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