Lipophorin as a yolk protein precursor in the mosquito, *Aedes aegypti*

Jianxin Sun, Tsuyoshi Hiraoka 1, Neal T. Dittmer, Kook-Ho Cho, Alexander S. Raikhel *

Department of Entomology, Michigan State University, East Lansing, MI 48824-1115, USA

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Abstract

We examined expression of the lipophorin (*Lp*) gene, lipophorin (*Lp*) synthesis and secretion in the mosquito fat body, as well as dynamic changes in levels of this lipoprotein in the hemolymph and ovaries, during the first vitellogenic cycle of females of the yellow fever mosquito, *Aedes aegypti*. Lipophorin was purified by potassium bromide (KBr) density gradient ultracentrifugation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Polyclonal antibodies were produced against individual *Lp* apoproteins, apolipoprotein-I (apo*Lp*-I) and apolipoprotein-II (apo*Lp*-II), with molecular weights of 240 and 75 kDa, respectively. We report here that in the mosquito *A. aegypti*, *Lp* was synthesized by the fat body, with a low level of the *Lp* gene expression and protein synthesis being maintained in pre- and postvitellogenic females. Following a blood meal, the *Lp* gene expression and protein synthesis were significantly upregulated. Our findings showed that the fat body levels of *Lp* mRNA and the rate of *Lp* secretion by this tissue reached their maximum at 18 h post-blood meal (PMB). 20-Hydroxyecdysone was responsible for an increase in the *Lp* gene expression and *Lp* protein synthesis in the mosquito fat body. Finally, the immunocytochemical localization of *Lp* showed that in vitellogenic female mosquitoes, this protein was accumulated by developing oocytes where it was deposited in yolk granules. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Lipophorin; Mosquito; Vitellogenesis; Fat body; Ovary; Gene expression; 20-Hydroxyecdysone; Yolk protein

1. Introduction

In insects, lipophorin (Lp) is the major hemolymph lipoprotein, which is composed of two apolipoproteins, 230–250-kDa apo*Lp*-I and 70–85-kDa apo*Lp*-II. A third small apoprotein (apo*Lp*-III) of 18–20-kDa is associated with Lp in a reversible manner. In a high-density lipoprotein (HDLp), one apo*Lp*-I and one apo*Lp*-II are associated with two apo*Lp*-III molecules (Kawooya et al. 1984, 1986; Ryan, 1990; Van der Horst, 1990; Surholt et al., 1992; Blacklock and Ryan, 1994; Soulages and Wells, 1994).

The main function of Lp is to transport lipids through the insect body, loading dietary lipids in the midgut and delivering them to the sites of their metabolism and storage. Lp also transports lipids from storage sites to utilization sites such as muscles (Kanost et al., 1990). Insect Lp carries mainly diacylglycerol and phospholipid (Van der Horst, 1990; Soulages and Wells, 1994).

A unique feature of insect Lp as a lipid delivery vehicle is its ability to function as a reusable lipid shuttle. In response to a stimulatory signal, resulting from an increased energy requirement, such as sustained flight, HDLp receives a diacylglycerol load from the fat body, which serves as the main lipid storage depot. The increase in lipid load results in the transformation of HDLp into a low-density lipophorin (LDLp). In the course of LDLp formation, a variable number of apo*Lp*-III molecules is added to the HDLp. At the site of delivery, the LDLp molecule unloads its lipid and releases apo*Lp*-III molecules, transforming itself back to HDLp. Importantly, the lipid loading and unloading of Lp molecules occurs without their internalization by either
donor or recipient cells. Thus, this mechanism allows an insect to meet an increased requirement for lipid transport without additional change in the Lp hemolymph concentration (Wells et al., 1987; Shapiro et al., 1988; Gondim et al., 1992; Ryan, 1996).

Oocyte development in insects involves the accumulation of large amounts of lipid, most of which is extra-ovarian in origin and is delivered by Lp; vitellogenin (Vg) contributes only about 5% of the oocyte lipid. A dual mechanism accounts for the Lp-mediated lipid delivery into the developing oocytes. The major vehicle for lipid delivery is the LDLp particle; however, some lipid is delivered by HDLp, which is internalized by developing oocytes via receptor-mediated endocytosis. Internalized HDLp, stripped of most of its lipid and apoLp-III molecules, is converted to a very high-density lipophorin (VHDLp), which is deposited in developing oocytes (Kawooya and Law, 1988; Kawooya et al., 1997). In both studies, however, determinations of Lp levels have been performed in whole bodies of mosquitoes. Therefore, in order to understand the role of lipophorin in development of mosquito oocyte, further studies should take into account the complexity of the physiological state of vitellogenic female mosquitoes.

In the present study, we analyzed in detail the expression of the Lp gene, Lp synthesis and secretion in the mosquito fat body, as well as dynamic changes in levels of this protein in the hemolymph and ovaries during the first vitellogenic cycle of A. aegypti females. We also investigated the regulation of the Lp gene by 20-hydroxyecdysone (20E) in the fat body. Finally we localized the Lp synthesis and accumulation by immunocytochemistry. Our findings showed that the fat body levels of Lp mRNA and the rate of Lp secretion by this tissue reached their maximum at 18 h PMB and the ovaries accumulated Lp where it was deposited in yolk granules of developing oocytes.

2. Materials and methods

2.1. Insects

Mosquitoes, A. aegypti, were reared as described by Hays and Raikhel (1990). Three to five days after eclosion, adult females were allowed to feed on white rats to initiate vitellogenesis. All dissections were performed in Aedes physiological saline (APS) (Hagedorn et al., 1977) at room temperature.

2.2. Materials

All analytic grade chemicals and protease inhibitors were purchased from Sigma and Calbiochem, respectively, unless stated otherwise. 32P-dATP (3000 Ci/mmol) for labeling nucleotide probes, 35S-sulphur reagent for protein labeling in vitro and 35S-methionine (1120 Ci/mmol) for fat body culture in vitro were from NEN Life Technologies, Inc. DEAE-sepharose CL-6B was from Pharmacia. Protein Assay reagent, Protein A-sepharose for immunoprecipitation and molecular weight standards for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) were purchased from Bio-Rad. Safety Solve II scintillation cocktail was supplied by Research Products International. RNA ladder (0.24–9.5 kb) was purchased from Life Technologies, Inc.

2.3. Lipophorin purification and antiserum preparation

Lipophorin was obtained from 2-day-old pupae. The pupae were washed with APS several times and homogenized in APS containing several protease inhibitors: 2

A. aegypti Lp concentrations increase upon ingestion of a blood meal, when the mosquito needs an increased rate of lipid transport to the developing ovaries. It has been reported by two laboratories that lipophorin reaches its maximal levels by 40–48 h post-blood meal (PBM) when major events of egg yolk and lipid deposition have been completed (Capurro et al., 1994; Van Heusden et al., 1997). In both studies, however, determinations of Lp levels have been performed in whole bodies of mosquitoes. Therefore, in order to understand the role of lipophorin in development of mosquito oocyte, further studies should take into account the complexity of the physiological state of vitellogenic female mosquitoes.

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μM pepstatin (Roche Molecular Biochemicals), 4 μg/ml each leupeptin, chymostatin and antipain, 10 μg/ml aprotinin, 5 mM e-amino-n-caproic acid (ACA), 1 mM benzamidine, 12.5 mM EDTA and 1.0 mM phenylmethylsulfonylfluoride (PMSF) (Roche Molecular Biochemicals). The homogenate was centrifuged briefly and the clear supernatant was subjected to a potassium bromide (KBr) density gradient ultracentrifugation as described previously (Pennington et al., 1996). Partially purified Lp fractions were further separated with 8% SDS–PAGE, and then specific Lp bands (apoLp-I and apoLp-II) were excised from the gel. The two different apoproteins were separately injected into rabbits with complete adjuvant. The specificity of the sera was tested by immunoblotting, using peroxidase-labeled goat anti-rabbit IgG (Cappel Organon Teknika Corp.) as second antibody. 

2.4. Fat body incubation in vitro and immunoprecipitation

Abdominal walls with adhering fat body from blood-fed or 3–5-day-old previtellogenic females were isolated and placed in a tissue culture system as described previously (Raikhel et al., 1997). To investigate the changes of Lp during vitellogenesis, synthesized and secreted Lp from fat body was radiolabeled with 35S-methionine as described above (Section 2.4). Lp purified from pupae was labeled with 35 S-methionine metabolically labeled Lp (specific activity, 38003.3 cpm/μg) was added to the homogenate to serve as a reference for Lp purification. Lipophorin was then purified with density gradient ultracentrifugation and the total Lp in the ovary extract was estimated by adjusting the amount of Lp measured to the percent of labeled Lp recovered.

SDS–PAGE was performed using either 8% or 6–15% gradient gels by the method of Laemmli (1970). Proteins were visualized by staining with either Coomassie Brilliant Blue R-250 or were processed for fluorography. Protein concentration was measured by Bio-Rad protein assay regent according to the instruction using BSA as a protein standard.

2.6. In vitro protein labeling

Lipophorin synthesized by fat body cultures was labeled with 35S-methionine as described above (Section 2.4). Lp purified from pupae was labeled with 35 S-sulfur labeling regent SJ440 as suggested by the manufacturer. Briefly, 50 μl (50 μCi) of SJ440 were aliquoted into an Eppendorf tube and a steady stream of nitrogen gas was blown across the top of the tube to evaporate the benzene in the solution. Next, 250 μg of protein dissolved in 100 mM sodium borate buffer (pH 8.6) were added and incubated on ice for 30 min. The reaction was stopped by the addition of 100 μl 200 mM glycine in the above borate buffer. The reaction mixture was separated by chromatography through a PD-10 column equilibrated in borate buffer.

2.7. RNA isolation and Northern blot analysis

RNA was isolated from female mosquito fat bodies by two methods. To examine the expression profile of the lipophorin gene, mRNA was purified from mosquito fat bodies dissected at selected time points pre- and post-blood meal utilizing the QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech Inc.). Total RNA from the fat bodies in culture was extracted with TRIZOL®
LS Reagent (Molecular Research Center, Inc.) according to the instructions from the manufacturer. Twenty-five to 35 fat body equivalents of mRNA or 20 μg of total RNA were fractionated by electrophoresis in 1% agarose/formaldehyde gel and transferred to a nitrocellulose membrane (Hybond) by conventional capillary blotting. A 1.0-kb fragment of the lipophorin gene was amplified from the genomic DNA by the polymerase chain reaction (PCR) described previously (Bej et al., 1991) and used as a probe. The primers for PCR were designed based on the partial nucleotide sequence of A. aegypti lipophorin cDNA (Van Heusden et al., 1998). The primer sequences were: upstream primer, 5′-CTTTGACTGCCGTGCTCCACGATC-3′; downstream primer, 5′-GAAAGTCCAAGGGAAATTGGTTGGTG-3′. PCR was conducted for 30 cycles of 94°C for 1 min, 68°C for 1 min, and 72°C for 2 min. The amplified fragment showed an expected size and nucleotide sequence. Random-primed probes were prepared with a DNA labeling kit (Boehringer Mannheim). The membranes were prehybridized in 50% formamide, 5× SSC (saline-sodium citrate), 50 mM sodium phosphate (pH 6.7), 100 μg/ml salmon sperm DNA and 5× Denhardt’s solution at 42°C for 4 h and hybridized with 32P-dATP labeled lipophorin DNA probe. The hybridized membranes were then extensively washed three times with 2× SSC, 0.1% SDS for 10 min at 42°C, and three times with 0.1× SSC, 0.1% SDS for 20 min at 65°C. Finally, the membranes were exposed to Kodak films at −80°C. The same membranes were stripped with two washes in 0.1× SSC, 0.05% SDS for 2 min at 95°C and rehybridized with mosquito actin (Deitsch et al., 1995) and/or vitellogenic carboxypeptidase (VCP) (Cho et al., 1991) DNA probes.

2.8. Immunocytochemical localization of lipophorin

Immunocytochemical observations using A. aegypti apoLp-I monoclonal antibody and a mixture of monoclonal antibodies specific against small Vg subunits were conducted to localize Lp, Vg and Vitellin in the fat bodies and ovaries. Cryosections of 6–8 μm were applied to Poly-prep™ coated slides (Sigma), and processed by the method of Raikhel and Lea (1983). The results were visualized by fluorescent photomicroscopy using a Zeiss Axioskop microscope equipped with phase contrast and epifluorescence, and Zeiss 210 laser scanning microscope, and recorded on Kodak film.

3. Results

3.1. Purification and characterization of mosquito lipophorin

A. aegypti Lp was purified from secretions of the vitellogenic fat body cultured in vitro and from pupae by the KBr density gradient ultracentrifugation (Fig. 1A). The KBr density gradient ultracentrifugation showed that Lp from these two sources possessed the same density (data not shown). The SDS–PAGE analysis confirmed that Lp secreted from the vitellogenic fat body cultured in vitro had a similar size to the lipophorin purified from mosquito pupae. In both cases, the mosquito Lp was composed of two apoproteins: the 240-kDa apoLp-I and the 75-kDa apoLp-II (Fig. 1B). We prepared polyclonal antibodies against each apoprotein, apoLp-I and apoLp-II. Immunoblotting analysis showed that these antibodies were specific to their respective Lp apoproteins (Fig. 1C). In all subsequent assays, we utilized the anti-apoLp-I antibody for detecting mosquito Lp.

3.2. Kinetics of lipophorin secretion by the mosquito fat body during the first vitellogenic cycle

To investigate changes in the rate of Lp secretion in the mosquito fat body during vitellogenesis, the fat bodies were cultured in vitro in a pulse-chase manner in a culture medium supplemented with either 35S-labeled or cold methionine, respectively. Fat bodies from pre- and postvitellogenic females collected at different times PBM with 6-h intervals, and were incubated as described in Section 2.4. The radiolabeled secreted proteins were collected from the chase media (Fig. 2A, lane M). Vg was separated by DEAE-Sepharose (Fig. 2A, lane 1). Lipophorin was immunoprecipitated by anti-apoLp-I antibodies (Fig. 2A, lane 3), and its levels were measured by radioimmunoassay (Hays and Raikhel, 1990). Vg was immunoprecipitated using a mixture of monoclonal antibodies against the Vg small subunit, and its levels were measured by the same method as Lp. The levels of total protein secreted by these fat bodies were also measured. In contrast to Vg, synthesis and secretion of which were initiated by a blood meal, the previtellogenic fat bodies produced Lp at a relatively high level (Fig. 2B). However, the rate of Lp secretion significantly increased after a blood meal, reaching a peak at 18 h PBM, and decreased thereafter to the previtellogenic levels by 30 h PBM (Fig. 2B). The rate of Vg synthesis and secretion exhibited much more dramatic PBM activation than that of Lp, reaching its peak at 24 h PBM but then dropping to background levels by 36 h PBM (Fig. 2B).

3.3. Changes in lipophorin levels in the fat body, hemolymph and ovary during the first vitellogenic cycle

Lipophorin levels in the fat body, hemolymph and ovaries were examined at 6 or 12 h intervals using the immunoblot analysis. Aliquots of the fat body culture medium, the hemolymph, the fat body and the ovary extracts were collected at each time point after a blood meal. The protein samples were separated with 6–15%
gradient SDS–PAGE and transferred onto a nitrocellulose membrane, and then detected with the apoLp-I specific antibody. Fig. 3A showed that Lp levels in the hemolymph gradually increased after the blood meal, reaching a peak at 18 h PBM, and then decreased to the previtellogenic level by 42 h PBM. Interestingly, the concentration of Lp in the fat body continued to increase, reaching its maximum at 36 h PBM, suggesting the possibility of reabsorption of Lp by the fat body from the hemolymph during postvitellogenesis (Fig. 3B). The previtellogenic and early vitellogenic ovaries had no detectable Lp; it was first detected at 6–12 h PBM and then increased gradually until 48 h PBM. These results suggested that following a blood meal, the Lp secreted into the hemolymph was probably sequestered by developing oocytes (Fig. 3B).

To further demonstrate the identity of ovarian Lp, we purified Lp from ovaries at 48 h PBM by density gradient ultracentrifugation, analyzed it using SDS–PAGE and Western blotting with apoLp-I and apoLp-II polyclonal antibodies. These analyses showed that ovarian Lp was similar to that from the fat body culture medium and consisted of apoLp-I and apoLp-II (Fig. 4). Preliminary quantitative analysis showed that at the time of termination of protein uptake (48 h PBM), Lp was 3% of the total ovarian protein (data not shown).

### 3.4. Expression of the mosquito Lp gene during the first vitellogenic cycle

To further investigate expression of the Lp gene, the Lp mRNA levels were examined by Northern blot analysis in fat bodies at different times PBM. During the previtellogenic period, the 10-kb pro-Lp transcript increased to relatively high levels by 3–5-days post-eclosion (Fig. 5A). Following a blood meal, the transcript levels started
Fig. 2. Changes of Lp synthesis rate in the female fat body during vitellogenesis. (A) Removal of vitellogenin (Vg) from the fat body culture medium using DEAE-Sepharose: M, the proteins secreted by the fat body; 1, Vg-bound fraction; 2, unbound fraction; 3, lipophorin, immunoprecipitated by the protein A-agarose–anti-apoLp-I antibody complexes from the unbound fraction. $^{35}$S-Methionine labeling and fluorography. (B) Radioimmunoassay determination of Lp, Vg and total protein secreted by the fat body at different times after a blood meal. The values are means±SE (n=3). Arrows point to the scales used for the proteins indicated.

to increase between 3 and 6 h PBM and reached peak levels between 12 and 18 h PBM, before dropping to a barely detectable level by 36 h PBM (Fig. 5B,C). VCP cDNA, reverse transcribed from the gene encoding a second yolk protein precursor in the mosquito, was used as a vitellogenic control in hybridization to the preparations of the same tissues (Fig. 5). The VCP gene was not expressed in the previtellogenic period, its transcript became detectable at 6 h PBM, reached its peak expression at 24 h PBM and then declined to undetectable levels after 36 h PBM (Fig. 5). Neither the VCP nor the Lp gene was expressed in the ovary (not shown).

3.5. 20-Hydroxyecdysone increases the Lp gene expression and protein synthesis in cultured previtellogenic fat bodies

Previtellogenic fat bodies from 3–5-day-old females were incubated for 6 h at 27°C in the culture medium in the presence or absence of $10^{-6}$ M 20E. In these
experiments, we also monitored the effect of 20E on production of Vg as a positive control. Culture medium from each sample was analyzed by Western blotting, utilizing polyclonal antibodies against apoLp-I and a mixture of monoclonal antibodies against the Vg small subunit as primary antibodies. The previtellogenic fat bodies secreted a considerable amount of Lp, but not Vg. 20-Hydroxyecdysone increased the Lp production in a dose-dependent manner similar to the response of the Vg gene to 20E; however, this enhancement of Lp production was not as dramatic as that of Vg (Fig. 6A).

The effect of 20E on the Lp gene was also examined at the mRNA level. The VCP gene was used as a positive control for induction by 20E. In contrast to VCP, Lp mRNA was present in the previtellogenic fat bodies. The levels of both VCP and Lp transcripts increased in response to 20E in a dose-dependent manner (Fig. 6B). However, the induction of the VCP gene and an increase in Lp mRNA levels by 20E in cultured fat bodies was blocked by addition of cycloheximide (Chx), a protein translational inhibitor, to the medium. The Chx inhibition was dose-dependent with $10^{-5}$ M Chx completely
abolishing the effect of $10^{-6}$ M 20E, which otherwise maximally increases $Lp$ gene expression (data not shown).

3.6. Localization of the synthesis and accumulation of $Lp$

Immunocytochemistry using the anti-apo$Lp$-I antibody and rhodamine-conjugated secondary antibodies revealed the distribution of $Lp$ in tissue sections (Fig. 7). In the fat bodies of vitellogenic females, this protein exhibited localization similar to that of Vg and VCP (Fig. 7B). However, in contrast to these yolk protein precursors, $Lp$ was also present in the previtellogenic and late-vitellogenic fat bodies (not shown).

In contrast to the fat body, no $Lp$ was found in the previtellogenic ovaries (not shown). In follicles of vitellogenic ovaries, $Lp$ was found in inter-follicular and the peri-oocytic spaces as well as in yolk bodies of oocytes (Fig. 7D,F). Vitellogenin, used as a positive control, appeared in similar compartment of the same follicles, but with much higher intensity of labeling (Fig. 7C,E).

4. Discussion

Our findings are in agreement with previous reports showing that in insects, including mosquitoes, the fat body is the site of $Lp$ synthesis and secretion. Likewise, we show that the mosquito apo$Lp$-I and apo$Lp$-II originate from the same $Lp$ precursor gene, expression of which leads to the $Lp$ transcript of 10 kb in size (Weers et al., 1992a,b; Sundermeyer et al., 1996; Van Heusden et al., 1998). However, we demonstrate here that in the mosquito $A. aegypti$, the fat body levels of $Lp$ mRNA and the rate of $Lp$ secretion by this tissue reached their maximum at 18 h PMB. Our conclusion rests on several lines of evidence which are in good correlation with each other: the rate of secretion of the newly synthesized fat body $Lp$ detected by the radioimmunoassay, the amounts of $Lp$ secreted by the fat bodies, the $Lp$ levels in collected samples of hemolymph detected by immunoblots, and finally, the $Lp$ transcript levels in the fat body, demonstrated by the Northern blot analysis. Our results are in agreement with those from previous studies in which $Lp$ was reported to reach its highest level at 40–48 h PMB (Capurro et al., 1994; Van Heusden et al., 1997). However, our study clearly showed the underlying complexity of the $Lp$ metabolism in the vitellogenic female mosquito, with $Lp$ being synthesized by the fat body and accumulated by the ovaries. Furthermore, the increased level of $Lp$ in the fat body at the postvitellogenic period suggests that this tissue internalized $Lp$ from the hemolymph after the completion of the yolk accumulation cycle by developing oocytes.

Our data show that the $Lp$ gene is expressed in pre- and postvitellogenic fat bodies. However, following blood feeding, the $Lp$ gene is upregulated, reaching its expression peak at 18 h PMB, at least 6 h before the peak expression of the yolk protein precursor genes vitellogenin ($Vg$), vitellogenic carboxypeptidase ($VCP$) and vitellogenic cathepsin-B ($VCB$) (Dhadialla and Raikhel, 1990; Hays and Raikhel, 1990; Cho et al. 1991, 1999). Despite this difference in expression of these genes, the
in vitro fat body culture experiments demonstrated that 20E is involved in elevating the Lp gene expression in a dose-dependent manner similar to that of Vg and VCP genes (Deitsch et al., 1995), with $10^{-6}$ M 20E to be required for maximal activation of all these genes. Furthermore, tests with the protein synthesis inhibitor, cycloheximide, show that similar to the Vg and VCP genes, the 20E activation of the Lp gene was completely inhibited by this reagent, indicating the requirement of protein synthesis for mediating the 20E regulation of these genes (Deitsch et al., 1995). Further analysis of regulatory regions of these genes should be conducted in order to elucidate the settled differences in regulatory circuitry of these genes determining the precise timing of their expression.

A blood meal taken by an anautogenous mosquito triggers a cascade of physiological events, which lead to rapid and synchronous development of oocytes into mature eggs ready for oviposition by 72 h PBM. The dramatic enlargement of oocytes in such a short period of time is a consequence of rapid accumulation of large quantities of extraovarian yolk protein precursors as well as lipid. In A. aegypti, three yolk protein precursors, Vg, VCP and VCB, are synthesized by the fat body and specifically accumulated by developing oocytes (Dhadialla and Raikhel, 1990; Hays and Raikhel, 1990; Cho et al. 1991, 1999). The data we report here, showing accumulation of Lp in developing oocytes, suggest that Lp also serves as a yolk protein precursor in this mosquito. Lipophorin has been implicated as a yolk protein precursor in eggs of lepidopteran insects, where it is deposited as VHDLp (Chino et al., 1977; Kawooya and Law, 1988; Kawooya et al., 1988; Telfer and Pan, 1988; Kulakosky and Telfer, 1990; Telfer et al., 1991).

In insect oocytes, accumulation of Vg is mediated by the specific Vg receptor (Sappington and Raikhel, 1998). The mosquito Vg receptor has been cloned and shown to represent a unique class of LDLp receptor family (Sappington et al. 1995, 1996; Sappington and Raikhel, 1998). Specific Lp receptors have been identified in the fat body of several insects (Tsuchida and Wells, 1990; Dantuma et al. 1996, 1997). More recently, cloning of the Locusta Lp receptor, homologous to the vertebrate VLDLp receptor, has been reported. The Locusta Lp receptor mediates Lp endocytosis by the fat body (Dantuma et al., 1999). The information concerning the Lp receptor in insect oocytes is limited. Osir and Law (1986) have reported that Manduca Lp does not compete with Vg for binding to oocyte membranes indicating that the internalization of these lipoproteins occurs via separate receptors. However, Kulakosky and Telfer (1990) have reported the competition between Vg and Lp during their internalization by Hyalophora vitellogenic follicle, suggesting the presence of a common receptor for both lipoproteins. Our preliminary data indicate that a specific Lp receptor exists in the mosquito oocytes, which is distinct from the Vg receptor and is homologous to the Locusta fat body Lp receptor and to the vertebrate VLDLp receptor (Sook-Jae Seo, Hyang-Mi Jun and Alexander S. Raikhel, unpublished observation).

In conclusion, we found that in the mosquito A. aegypti, Lp is synthesized by the fat body, with a low level of the Lp gene expression and protein synthesis being maintained in pre- and postvitellogenic females to meet general metabolic and developmental needs. Following a blood meal, the Lp gene expression and protein synthesis are significantly upregulated, reaching their peak levels at 18 h PBM. 20-Hydroxyecdysone is responsible for increased expression of the Lp gene and protein synthesis in the mosquito fat body. Immunocytochemical data reveal that some of the newly produced Lp is accumulated by developing oocytes as a yolk protein precursor. Our preliminary results demonstrate that Lp
constitutes only 3% of total ovarian proteins after the completion of protein accumulation. Considering that lipids make up approximately 35–40% of the insect egg dry weight (Kawooya and Law, 1988; Briegel, 1990), internalization of Lp is unlikely the major route of lipid delivery to the developing oocyte. Thus, elucidation of the precise role of Lp as a yolk protein precursor in the mosquito oocyte requires further studies.

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