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Differential partitioning of maternal fatty acid and phospholipid in neonate mosquito larvae

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Summary

In animals, lipids are a source of energy, cell membrane components, signaling pathway modulators and emulsifying agents. In egg-laying animals, maternal yolk lipids, imported into the egg before laying, are metabolized or distributed in the developing embryo to serve these functions. Studies with birds, reptiles and insects have described lipid metabolism in adults and in eggs, but no studies have addressed how lipids are distributed in developing organs in the embryo. Here we show that maternal fatty acid and phospholipids segregate differently in tissues of newly hatched mosquito larvae. In the mother, both lipids are colocalized in yolk granules of developing oocytes and distributed evenly. In neonate larvae, however, the maternal fatty acid is stored along the side of the body, especially at the base of the body hair, and in the thorax, where the muscles are located, probably to provide energy for the rapid movements needed to find food immediately after birth. Most maternal phospholipids, however, are concentrated in the motile intestinal gastric caeca, from which they are released into the gut lumen where they may act as emulsifiers, probably to facilitate assimilation of the food the neonate ingests. Similar phenomena were observed in both *Anopheles gambiae* and *Aedes aegypti* mosquitoes, suggesting that such differential segregation of lipids is common to both insects. This study may lead to improved delivery of larvicidal agents and to efficient killing of newly hatched mosquito larvae as a control strategy for mosquito-borne diseases.

Key words: Malaria vector, mosquito, lipid metabolism, lipophorin, fatty acid, phospholipid, neonate.

Introduction

Animals need maternal resources during embryonic development and immediately after birth. In live-bearing animals, mothers are able to reduce the investment of nutritional resources in the egg and compensate by supplying nutrients to the developing embryo *via* the placenta during gestation, when the fetus is continuously nourished by the unlimited supply of maternal nutrients (Speake and Thompson, 2000). In these animals, glucose is the primary source of energy for fetal development. In egg-laying animals such as birds (Noble and Speake, 1997; Speake et al., 1998), reptiles (Speake and Thompson, 2000) and insects, developing embryos have access only to those nutrients imported to the egg before laying. In these animals, lipids are the major source of energy of the eggs. Therefore, for optimum utilization, the imported lipids in eggs need to be properly stored and made available when they are needed during embryogenesis and immediately after birth.

Studies with chicken eggs described lipid metabolism in the developing embryo (Speake et al., 1998; Noble and Cocchi, 1990). During development, especially in the second half, there is an increased lipid transfer from the yolk to the embryo (Noble and Cocchi, 1990). Lizards are also an interesting model for studying lipid transport to offspring, because in this family there are both live-bearing and egg-laying species (Speake and Thompson, 2000). Lipid composition in eggs from alligators (Speake and Thompson, 1999) and from some turtles (Rowe et al., 1995) have also been studied.

Studies with such insects as *Manduca sexta* and *Locusta migratoria* have described insect lipid transport processes (Ryan et al., 1986; Arrese et al., 2001). Lipid is carried by a lipoprotein complex, called lipophorin, which picks up lipid from the gut and delivers it to tissues such as developing eggs (Shapiro et al., 1984; Kawooya et al., 1988; Gondim et al., 1989; Atella et al., 1992, 1995). In *M. sexta*, approximately 40% of the dry mass of the mature egg is lipid. Most of these lipids are imported from the maternal lipid pool, biosynthesis accounting for only about 1% of the lipid in the egg (Canavoso et al., 2001).

Among the lipids, triacylglycerols consist of three molecules of fatty acids and are the most concentrated form of energy

present in the yolk. Phospholipids and free cholesterols are poor sources of energy. Phospholipids are major components of cell plasma membranes. Because lipids serve functionally different purposes, segregation of lipids in appropriate tissues in embryos of egg-laying animals is essential for development of healthy neonates. However, little is known about how imported lipids are distributed during embryogenesis and in neonates.

Unlike imported proteins and nucleic acids that usually degrade to monomers before utilization, most imported lipids remain intact and can be easily traced in live animals. Farber et al. (2001) used fluorescently labeled phosphatidylcholine substrate to trace phospholipase enzyme in live zebrafish, where labeled lipids accumulated in the digestive organs and the gall bladders that fluoresced intensely (Hendrickson et al., 1999; Farber et al., 2001). In the present work, we used fluorescently labeled fatty acids and phospholipids to investigate maternal lipid uptake and distribution, both in the developing egg and in the neonate mosquitoe larvae. Because mosquitoes need blood feeding to trigger egg development (Clements, 1992), they provide an opportunity to monitor of a batch of synchronously developing oocytes with precision. Our results show a novel differential partitioning of fatty acid and phospholipid in the newly hatched larvae.

Materials and methods

Mosquitoes and materials

Anopheles gambiae Giles (G3 strain) and *Aedes aegypti* L. (Rockefeller strain) mosquitoes were maintained under standard insectary conditions (27°C and 80% relative humidity, 12.00:12.00 L:D photoperiod) and fed on a cotton ball saturated with diluted Karo syrup (CPC International Inc., Englewood Cliffs, NJ, USA). For blood feeding, 6–8 days after emergence from pupae, adult female mosquitoes were starved overnight and fed on 4- to 5-week-old White Leghorn chickens. Fed mosquitoes were kept in the insectary until used. All fluorescently labeled fatty acids and phospholipids were purchased from Molecular Probes (Eugene, OR, USA).

Purification of lipophorin

Mosquito lipophorins were purified using potassium bromide gradient ultracentrifugation as described earlier (Gondim et al., 1989). Mosquitoes (4 g) were homogenized using a glass hand grinder (Thomas Scientific, Swedesboro, NJ, USA) on an ice bath in the presence of 20 mmol l^{-1} glutathione, 5 mmol l^{-1} EDTA, 2 mmol l^{-1} PMSF, 0.5 μg $μl^{-1}$ antipain, 5 μmol l^{-1} pepstatin and 0.5 μg $μl^{-1}$ leupeptin in PBS (10 mmol l^{-1} sodium phosphate, 0.15 mol l^{-1} NaCl, pH 7.4). The homogenate was then centrifuged (100 000 ***g***, 30 min, 4°C) to remove insoluble material. Solid potassium bromide was added to the supernatant to a final concentration of 0.4 g ml^{-1}, and the mixture was again centrifuged at 125 000 ***g*** in a Beckman 50.2 Ti rotor at 4°C for 20 h. The gradient was then fractionated from top to bottom, and lipophorin fractions were pooled, extensively dialyzed against PBS, and stored under liquid nitrogen until use. The degree of purification was monitored by SDS-PAGE, and protein concentration was estimated using a microBCA kit (Pierce, Rockford, IL, USA) in the presence of 0.5% SDS, using bovine serum albumin as standard.

Preparation and measurment of ^{32}P-phospholipid-labeled lipophorin

Into the hemolymph of adult female *A. gambiae*, 0.1 μl of ^{32}Pi (1 μCi, 3700 Bq, Amersham, Piscataway, NJ, USA) was injected using a PLI-100 microinjector (Harvard Apparatus, Holliston, MA, USA). 1 day later, ^{32}P-phospholipid-labeled lipophorin (^{32}P-lipophorin) was purified from the total homogenate of the injected mosquitoes using potassium bromide gradient ultracentrifugation as described above (Gondim et al., 1989). ^{32}P-labeled lipophorin was injected into mosquito hemolymphs at different days after a blood meal using a PLI-100 microinjector. Ovaries were dissected, separated according to the stages described by Christophers (1911), homogenized, and the radioactivity measured by scintillation counting.

In vitro *labeling of lipophorin with fluorescent lipid analogs*

Purified lipophorin was labeled with Texas Red-conjugated phosphatidylethanolamine, N-Texas Red® sulfonyl-1,2-dihexanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (Texas Red-PE), BODIPY-labeled palmitic acid, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-hexadecanoic acid (BODIPY-FA), or BODIPY-labeled phosphatidyl choline, 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine (BODIPY-PC) (Molecular Probes) as described by Martin-Nizard et al. (1987) with slight modifications. Briefly, fluorescent lipid-coated glass beads (0.2 mg) were incubated with 2 mg of pure lipophorin. The mixture was gently stirred at room temperature for 1 h, and the glass beads removed by centrifugation (5 min at 9500 ***g***). Fluorescently labeled lipophorin was re-isolated by gel chromatography on a column of Sephacryl S-200 concentrated by Microcon filters (Amicon, Bedford, MA, USA). Lipophorin was also labeled with fluorescein 5′-isothiocyanate using a Fluorescein-EX Protein labeling kit (FluoReporter®; Molecular Probes).

Observation of fluorescent lipids in live oocytes and larvae

2 days after a blood meal, fluorescently-labeled lipophorin was injected into the hemolymph of vitellogenic females. 3 days later, egg laying was induced by transferring the mosquitoes to a Petri dish with a 80 mm filter paper disk soaked with distilled deionized water. The Petri dish was protected from the light until the larval hatch. Approximately 36 h later, the larvae emerged from the eggs and were collected immediately and maintained in ice protected against the light. To visualize the lipids in live oocytes and larvae, a 0.5-mm thick plastic O-ring sticker was placed on a glass microscope slide, into the middle of which dissected oocytes or newly hatched larvae were placed and covered with a coverslip. The

O-ring protects the oocytes and larvae from being damaged by the coverslip. The slide was immediately transferred to the microscope stage for observation. Fluorescence was analyzed by a confocal microscopy (Leica DMIRBE microscope, Leica TCS-NT/SP confocal; Leica Microsystems GmbH, Heidelberg, Germany) equipped with argon laser. Additional observations were made by epifluorescence microscopy (Carl Ziess, Inc., Thornwood, NJ, USA) with monochromatic filters for BODIPY (excitation 505 nm/emission 515 nm) or for Texas Red (excitation 582 nm/emission 601 nm). High-resolution digital images were captured and processed using Adobe Photoshop (Adobe Systems, Inc., San José, CA, USA).

Thin-layer chromatography of lipids

Lipid extraction was performed according to Blight and Dyer (1959) for 2 h in a stoppered tube in 5 ml of chloroform–methanol–water solution (2:1:0.8, v/v) with intermittent agitation. After centrifugation, the supernatant was collected and the precipitate subjected to a second lipid extraction (1 h). To the pooled extract, 5 ml of water and 5 ml of chloroform were added; the mixture was shaken and, after centrifugation, the organic phase was removed and dried under nitrogen. The amount of total lipid was determined gravimetrically. Extracted lipids were analyzed by thin-layer chromatography as described for phospholipids (Horwitz and Perlman, 1987) on silica gel plates (Merck KGaA, Darmstadt, Germany). Lipids were visualized by illuminating the plates with a UV lamp.

Results

Uptake and distribution of lipid by Anopheles gambiae *oocytes*

To examine lipid transfer to oocytes *in vivo*, we injected radiolabeled (^{32}P) lipophorin into the hemolymph of vitellogenic female mosquitoes. Ovaries were excised from these mosquitoes at different stages of development to measure lipophorin uptake. The uptake of lipophorin was maximum around Christophers stage 2 and 3, approximately day 2 after the blood feeding (Fig. 1A). We also incubated excised ovaries from mosquitoes not injected with radiolabeled lipophorin and incubated them *in vitro* with radioactive iodine (^{125}I)-labeled lipophorin. In agreement with *in vivo* results, the rate of uptake with the incubated ovaries on day 2 was maximum and linear with incubation time (data not shown).

To examine whether the entire lipophorin complex is taken up or only lipids are delivered as the lipophorin binds to the oocyte, we labeled the apolipoprotein moiety of purified lipophorin with fluorescein isothiocyanate. The fluorescent

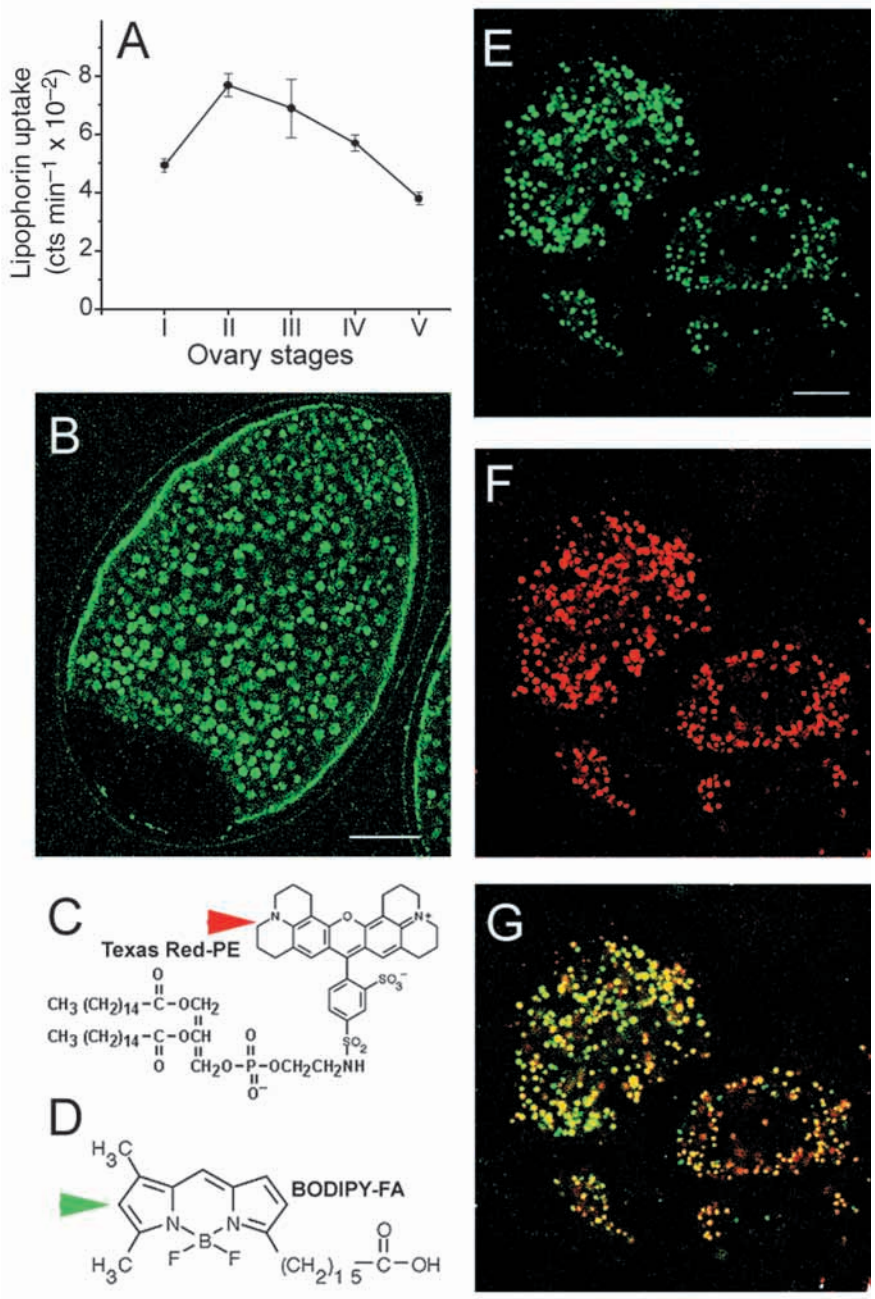

Fig. 1. Uptake and distribution of lipophorin, fatty acid and phosphatidylethanolamine by the ovary and oocyte of the mosquito *Anopheles gambiae* strain G3. (A) Uptake of ^{32}P-labeled lipophorin by different stages (Christophers, 1911) of the developing ovary. (B) Localization of FITC-labeled maternal lipophorin in developing oocytes. Scale bar, 25 μm. (C) Phosphatidylethanolamine analog labeled with Texas Red (Texas Red-PE, excitation 582 nm/emission 601 nm) on the polar head. (D) Fatty acid analog labeled with BODIPY (BODIPY-FA, excitation 505 nm/emission 515 nm) on the acyl chain. (E) Localization of the fatty acid (BODIPY-FA) in *A. gambiae* oocytes. Scale bar, 15 μm. (F) Localization of phospholipid (Texas Red-PE) in the same oocyte as in E. (G) Merged panels E and F demonstrate that fatty acids and phosphatidylethanolamine colocalized in the same vesicles.

lipophorin was injected into mosquito hemolymph. 2 days after injection, fluorescence was observed surrounding the follicular cell lining and in the micovillar region, similar to the sites where vitellogenin binding had been observed earlier (Sappington and Raikhel, 1998). Lipophorins were also found in spherical yolk bodies in the cortex, except in the nurse cell region (Fig. 1B). Similar distribution of lipophorin was observed earlier with *A. aegypti* oocytes (Sun et al., 2000).

Distribution of imported lipids in oocytes was also examined by labeling lipophorins with fluorescently tagged fatty acid and phospholipids. Purified lipophorin was loaded with a mixture of Texas Red-tagged phosphatidyl-ethanolamine (Texas Red-PE) and BODIPY-labeled palmitic acid (BODIPY-FA) (Fig. 1C,D) and injected into the hemolymph of blood-fed mosquitoes. When developing eggs from these mosquitoes were examined, both lipids were found in oocytes (Fig. 1E,F) colocalized within the same yolk vesicles with lipophorin (Fig. 1G), demonstrating that imported lipids are stored in the same compartment in developing oocytes.

Maternal phospholipid and fatty acid segregate differently in neonate larvae

To examine the imported maternal lipids in newly hatched mosquito larvae, eggs from the mosquitoes previously injected with lipophorin fluorescently labeled with phospholipid (Texas Red-PE; Fig. 1C) and fatty acid analog (BODIPY-FA; Fig. 1D) were collected. 2 days later when the eggs hatched, live larvae were examined for maternal lipid within 30 min after hatching. Both red and green fluorescence were found in the newly hatched larvae (Fig. 2), indicating that at least some of the imported lipids survived embryogenesis.

Distribution of the lipid types, however, was remarkably different in the larvae. With a setup of higher gain of fluorescence, fatty acids were seen in most parts of the body, but when gain was adjusted to a lower setting to examine the site of maximum accumulation, the majority of the fatty acid (BODIPY-FA) was found segregated in spherical vesicles in the thorax and along the side of the body cavity (Fig. 2A). Accumulation of fatty acid or phospholipids in head was remarkably low. Along the body, fatty acid-containing vesicles also concentrated at the base of

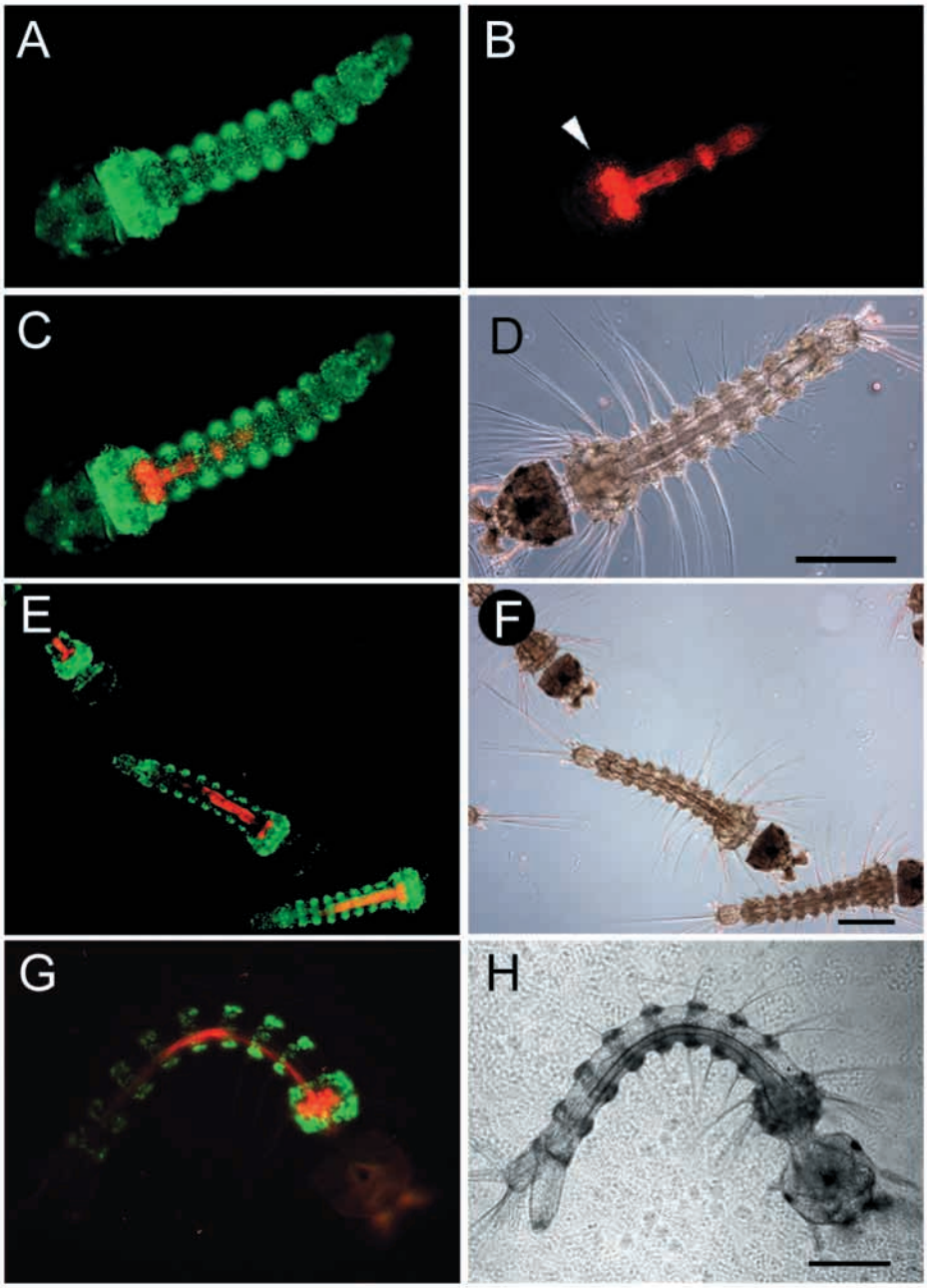

Fig. 2. Maternal lipid localization in neonate mosquito larvae. Purified lipophorin was double labeled with Texas Red-PE and BODIPY-FA before injecting into vitellogenic females; eggs were collected and kept in water at 28°C until larval hatching. The fluorescence associated with newborn larvae was examined within 30 min after hatching. (A) Localization of BODIPY-FA in *A. gambiae* larvae. (B) Localization of Texas Red-PE in the same newborn larvae as shown in A. (C) Merged image of A and B shows differential localization of BODIPY-FA and Texas Red-PE. (D) Phase-contrast image of the same larvae as shown in C. (E) All neonate larvae hatched from the same batch of eggs, showing identical pattern of differential partitioning of fatty acid (BODIPY-FA) and phosphatidylethanolamine (Texas Red-PE). (F) Phase-contrast image of the larvae shown in E. (G) Maternal lipid distribution in the larvae of *A. aegypti* mosquitoes. (H) Phase-contrast image of larvae shown in G. Scale bar, 200 μm.

the body segment hair. In contrast to the fatty acid, however, imported phosphatidylethanolamine was segregated differently and mostly found associated with the upper intestine and the gastric caeca. In mosquito larvae, gastric caeca are eight pouch-like structures located immediately behind the cardia and opening into the anterior end of the midgut (Volkmann and Peters, 1989). They are located within the thorax of the neonate larvae (Fig. 2B). In every larva, the gastric caeca was found to be filled with the phospholipid (Texas Red-PE). In the intestine, although the phospholipids were always seen near the upper gut region close to the gastric caeca, the length along the gut where fluorescence was found was variable. In most cases, this was limited to one third of the upper gut only, but phospholipids sometimes filled most of intestine from the gastric caeca to the lower midgut. Movement of the phospholipid from the caeca to intestine was also frequently observed. There was little overlap in the localization of stored fatty acids and phospholipids seen in the neonate larvae (Fig. 2C,D). The unique segregation of the fatty acid and the phospholipid was reproducible in all experiments (N=32) and in all larvae examined from each batch of eggs (Fig. 2E,F) obtained from labeled lipophorin-injected mosquitoes (15 mosquitoes in each experiments).

Similar partitioning of lipid also occurs in A. aegypti *larvae*

To investigate whether differential partitioning of lipids occurs in mosquitoes other than *Anopheles*, we injected Texas Red-PE- and BODIPY-FA-labeled lipophorin into the hemolymph of blood-fed *A. aegypti* mosquitoes (order Culicidae). *Aedes* was evolutionarily separated from *Anopheles* at least 150 million years ago (Kwiatowski et al., 1995). The geographic distribution of these mosquitoes is also different, and they are vectors of different blood-borne human pathogens (Lehane, 1991). Maternal lipids were seen in neonate larvae of both mosquitoes, suggesting that, similar to *Anopheles*, some maternal lipids also remain intact during *Aedes* embryogenesis. Furthermore, the fatty acid and phospholipid were distributed in *A. aegypti* larvae with a pattern similar to that seen in *A. gambiae* (Fig. 2G,H), showing that differential partitioning of phospholipid and fatty acid is a common phenomenon in neonates of both of these mosquitoes.

We then examined whether the fluorescence in the larvae is due to the accumulation of a metabolized form of Texas Red-PE instead of the intact molecule. For this, we extracted total lipids from the labeled neonate larvae and fractionated them on thin-layer chromatography. Fig. 3 shows that the phosphatidylethanolamine analogs from the larvae had identical relative mobility to that of the Texas Red-PE originally injected into the mosquito, which indicates that the fluorescence in the gastric caeca and intestine of neonate larvae was due to the intact phosphatidylethanolamine analog and probably not due to any metabolized fluorescent product.

Both phosphatidylcholine and phosphatidylethanolamine segregate in gastric caeca

To verify whether accumulation in the gastric caeca is unique to phosphatidylethanolamine or common to other phospholipids as well, mosquitoes were injected with lipophorin loaded with a mixture of two different phospholipids fluorescently tagged at different positions. The phosphatidylcholine analog (BODIPY-PC) was labeled with BODIPY at the gamma position of one of the fatty acid chains (Fig. 4A), and Texas Red-PE (Fig. 1C) was labeled with Texas Red moiety at the ethanolamine domain. When the newly hatched larvae from these mosquitoes were examined (Fig. 4D), phosphatidylcholine was found in the gastric caeca (Fig. 4B) along with the phosphatidylethanolamine (Fig. 4C). This demonstrated that both phospholipids accumulate at the same structure in the mosquito, which is different from the structure where fatty acid accumulates. Notably, some green fluorescence derived from BODIPY-PC, where BODIPY was attached to a fatty acid, was also found along the body with a pattern similar to the fatty acid distribution (Fig. 4B). Such fluorescence was not seen when the ethanolamine domain of PE was tagged with Texas Red. This suggested that the fatty acid moiety of some phospholipids is exchanged with other lipid forms during embryogenesis.

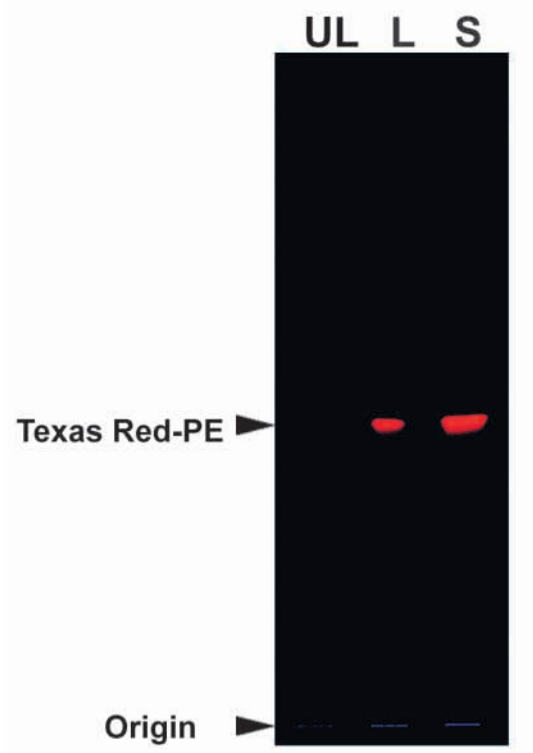

Fig. 3. Intact imported phosphatidylethanolamine in neonate *A. gambiae* larvae. Texas Red-PE-loaded lipophorin was injected into vitellogenic females. Extracted phospholipids from larvae were fractionated by thin-layer chromatography. Fluorescence from the lipid analog was visualized by illuminating the plate with UV light. Lane UL, unlabeled phospholipids extracted from unlabeled neonate larvae; lane L, labeled phospholipids extracted from neonate larvae hatched from eggs laid by the females injected with Texas Red-PE; lane S, standard containing commercial Texas Red-PE that was injected into the mosquitoes.

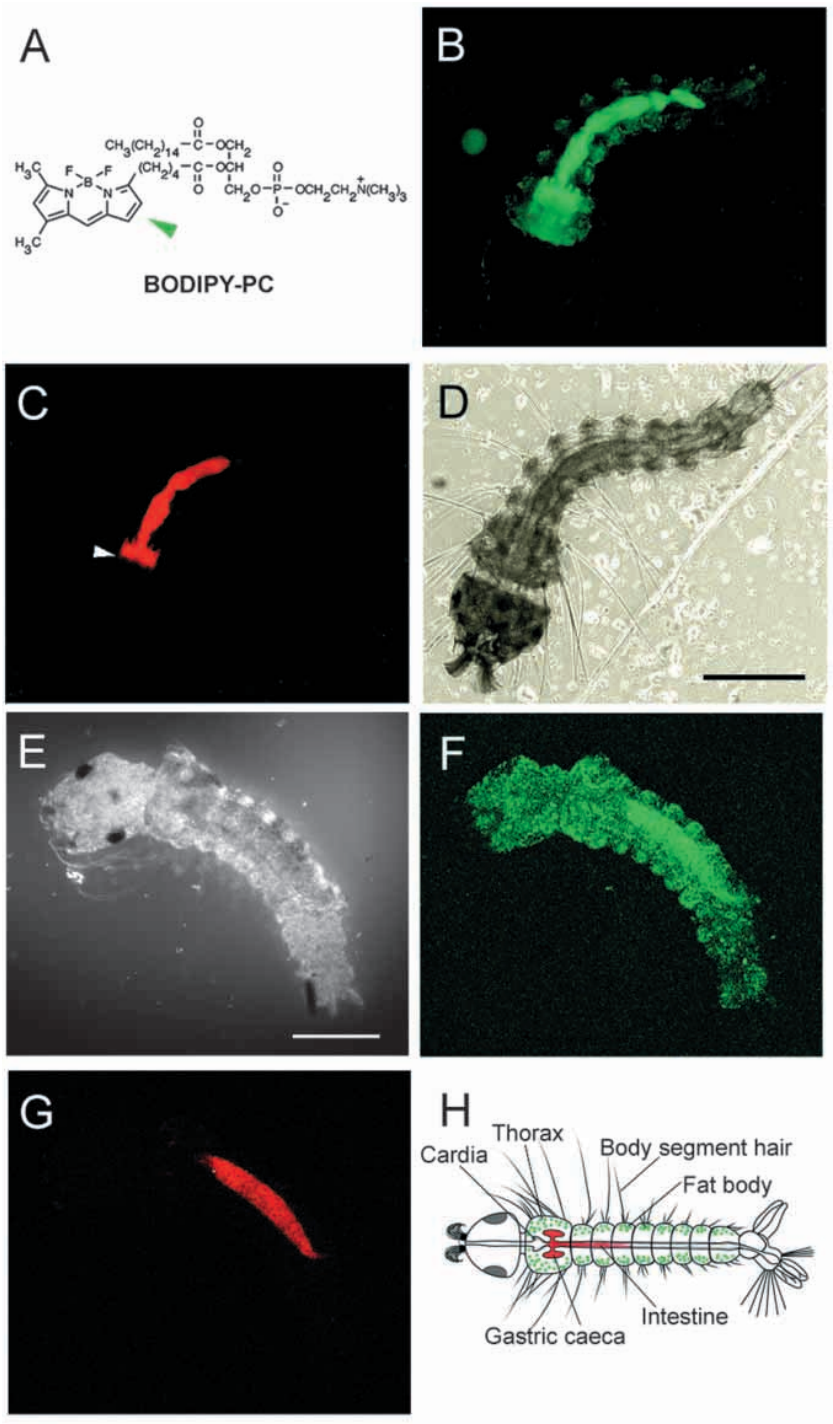

Fig. 4. Distribution of phospholipids and fatty acids in neonate and embryonic larvae. (A) BODIPY-labeled phosphatidylcholine analog (BODIPY-PC). (B) Location of phosphatidylcholine analog (BODIPY-PC) in newly hatched *A. gambiae* larvae. (C) Location of phosphatidylethanolamine analog (Texas Red-PE) in the same larvae. (D) Phase-contrast image of the larvae shown in B and C. Scale bar, 200 µm. (E) Phase-contrast image of approximately 30-h-old embryonic *A. gambiae* larvae. Scale bar, 150 µm. (F) Fluorescent image of the same larvae in E, showing the distribution of BODIPY-labeled maternal fatty acid (BODIPY-FA). (G) Image of the same embryonic larvae showing the distribution of the Texas Red-labeled maternal phosphatidylethanolamine (Texas Red-PE). (H) Diagram of a neonate *A. gambiae* larvae depicting the major accumulation sites of the maternal phospholipids (red) and fatty acids (green).

Partitioning of the maternal lipids occurs during embryogenesis

To investigate the process of lipid segregation and to determine the stage at which the maternal lipid partitioning occurs in the larvae, we examined live developing embryos dissected from the eggs laid by mosquitoes previously injected with Texas Red-PE and BODIPY-FA. At 26°C and 80% relative humidity, it takes approximately 36 h for *A. gambiae* eggs to hatch. We dissected eggs approximately 30 h after laying to obtain intact embryonic larvae (Fig. 4E). At this stage, most larval structures were formed and easily identifiable, although these premature larvae were not motile. When examined for lipid distribution, fatty acids were found distributed in the entire body of the embryonic mosquito larvae including the developing head (Fig. 4F). The phospholipids, however, were confined within the intestine (Fig. 4G). Some fatty acids were also present in the developing intestine of the embryo with the phospholipids. This suggests that, although incomplete, the separation of the lipids occurs during embryogenesis prior to hatching of the mature larvae.

Discussion

We have observed a novel process of lipid partitioning in neonate mosquito larvae. Phospholipid labeled in either the fatty acid domain or the phosphatidyl domain segregated in the upper intestine and the gastric caeca of the larvae. In developing embryos dissected from eggs after approximately 30 h, phospholipids were found accumulated in the gut lumen; however, in newly hatched larvae, a major portion of maternal phospholipids was observed inside the gastric caeca, from which it appeared to be released into the gut. In contrast, fatty acids accumulate in droplets that segregate primarily in the thorax, where muscles are located, and along the body, specifically near the base of the body segment hair. Because in the newly hatched larvae the majority of phospholipids are completely separated from the fatty acids and always found in the gastric caeca, segregation of lipids may start during embryogenesis, but completes at the late stage of the development, or immediately after their emergence from eggs. We were unable to resolve the mechanism and pinpoint exactly when phospholipids are separated from fatty acids in the embryo.

Biologically active fluorescent lipids are commonly used to elucidate complex biological

phenomena in live animals. To track cells *in vivo*, surface-labeling fluorescent fatty acid analogues, such as PKH26, have been used (Horan et al., 1990; Teare et al., 1991). PKH26 was also used to track malaria parasite *P. gallinaceum* ookinetes in mosquitoes, to study parasite–vector interactions (Shahabuddin et al., 1998; Zieler et al., 1999). Fluorescent lipids also have been used to label intracellular organelles (Cole et al., 2000; Moreno et al., 2000; Buckman et al., 2001) and to locate lipids in live animals (Hendrickson et al., 1999; Farber et al., 2001). In this study, we have used fluorescently labeled lipids to examine maternal fatty acid and phospholipid transport and distribution in mosquito eggs and larvae.

Fatty acids and glucose are the primary sources of energy in animals. In exclusively egg-laying animals such as chickens and mosquitoes, the entire embryogenesis process takes place outside the mother's body; for these animals, fatty acids are the major energy source (Speake and Thompson, 2000). Phospholipids, however, play more complex roles: they are major components of the cell wall, precursors of cell signaling molecules (Payrastre et al., 2001), and biological lubricants (Hills, 2000) and food emulsifiers (Hasenhuettl and Hartel, 1997). Phospholipids also act as surfactants in normal mammalian pulmonary function (Veldhuizen et al., 2000). Phospholipids such as lecithin, when added to food, act as emulsifiers and enhance assimilation (Hasenhuettl and Hartel, 1997). Lecithins are a mixture of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol.

What roles might the uniquely segregated fatty acids and phospholipids play in neonatal larvae? They may be related to finding and ingesting the food that the newly born larvae need to survive immediately after hatching. Stored maternal fatty acids may provide the energy for the initial rapid movements needed for finding food. Fatty acid accumulation along the body and thorax, where muscles for these movements are located, makes it a convenient, ready-made energy source. Localization of the fatty acid-containing vesicles at the thorax and at the base of the body segment hairs also supports the possibility that energy required for rapid muscle contractions and hair movement in neonate mosquito larvae is supplied by the stored maternal fatty acids (Fig. 4H). This is corroborated by the observation that neonate larvae of both *A. aegypti* and *A. gambiae*, when left in distilled water, move about vigorously, slowing as the fluorescence of fatty acids is depleted (data not shown). The larvae eventually die if food is not provided at this stage. The first ingested food of neonate larvae also needs to be digested and absorbed quickly, with rapid dissolution of food particles. As mentioned above, phospholipids are excellent emulsifiers and lubricants that, when released into the intestine, may facilitate digestion of the first-ingested food particles.

We have therefore described a novel process for differential partitioning and storage of maternal fatty acids and phospholipids that may allow neonate mosquito larvae to deal with the first few hours of life. Better understanding of this mechanism may lead to development of more efficient insecticide delivery tools, where larvicidal toxins can be more effectively released and absorbed by larvae. This may contribute to improved strategies for controlling mosquito-borne diseases such as malaria.

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