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REGIONAL AND FUNCTIONAL DIFFERENTIATION IN THE INSECT FAT BODY[1]

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ABSTRACT

Insect fat body historically has been considered a single tissue with multiple and diverse metabolic functions. Recent findings indicate that functionally distinct regions of fat body exist in some insects and that these regions often have unique morphologies. Regionally differentiated fat bodies in Lepidoptera have been associated with the segregation of synthetic and storage activities. The loss of biosynthetic capacity for storage proteins at the beginning of metamorphosis is the consequence of the histolysis of a larval specific histotype, whereas the histotype that accumulates storage proteins persists into the adult stage. In Diptera, functional histotypes are arranged along the anterior-posterior axis and between the larval and adult stages. Based on these models, we predict that the use of immunological and molecular probes will lead to the identification of regionally and functionally differentiated fat body histotypes in many insects.

PERSPECTIVES AND OVERVIEW

Fat body often has been viewed as a singular tissue that performs a myriad of metabolic functions that change as the insect progresses through development (18, 34, 48, 49). The breadth of fat body metabolic activities encompasses intermediary metabolism and involves the homeostatic maintenance of hemolymph proteins, lipids, and carbohydrates (34). Fat body also performs developmentally specific metabolic activities that produce, store, or release components central to the prevailing nutritional requirements or metamorphic events of the insect.

Underlying the multiple metabolic functions associated with the fat body is a structural pleomorphism that has received only limited attention. Because the different cell types are typically mixed and integrated into a unified tissue, there has been little success in correlating a specific functional activity with the cell type in which it occurs. On a gross level, the allocation of stage-specific functions to specific cell types has been readily discernible in Diptera, in which the adult fat body is derived from histoblasts during metamorphosis and therefore differs from the larval fat body, which undergoes histolysis during metamorphosis (12, 67). The dipteran larval and adult fat bodies have specific functions related to the stage of development, e.g. the larval fat body produces larval serum proteins and the adult female fat body produces yolk polypeptides. On the other hand, the classical view for Lepidoptera is that they have one fat body tissue that undergoes remodeling from a larval to an adult form. Although some histolysis of the fat body during metamorphosis had been suggested (21), the change of function of the fat body during metamorphosis was ascribed to a transformation of cellular activity during reorganization (18).

However, recent findings indicate that the latter perspective must be reassessed. In two different families of Lepidoptera, two distinct types of fat body have been identified, on the basis of color differences, that clearly have different structures and functions. The fat body histotypes are tan and white in the Indianmeal moth, *Plodia interpunctella* (Pyralidae) (71), and blue and white in the corn earworm, *Helicoverpa zea* (Noctuidae) (25, 86). In both cases, the fat bodies before and after pupation differ in structure, development, and ability to accumulate storage proteins. Moreover, the fate of these histotypes during development is different. Because multiple functions and structural forms are associated with the fat body in many insect species, differentiation into separate tissue types that develop and function separately could be a more general phenomenon. On the other hand, some evidence suggests that the fat body is a single pleiomorphic tissue that shifts functional activity during development. The actual structural and functional relationships of fat body cell types probably lie between these two positions.

Historically, the resolution of these questions has not been a simple matter.

The integration of different cell types that change morphology and function during development within the fat body tissue makes working with this tissue difficult (18) and has complicated studies on it. In recent years, however, immunological and molecular biological probes have made associating specific functions with different structures within tissues easier. This review focuses on systems that are pertinent to establishing structure-function relationships in this tissue and gives a perspective to fat body tissue diversity. Only species of Diptera and Lepidoptera are discussed in detail, because little information on these topics has been published for other orders. The review emphasizes specific functional activities but does not extensively examine the general morphology and the central role of the fat body in intermediary metabolism, which have been described previously in excellent reviews (18, 34, 48, 49). From this information base, we attempt to provide the context for new research to delineate the nature of the insect fat body.

ORGANIZATION OF THE FAT BODY

The gross morphology of the fat body varies widely between different insect species. Generally, this tissue is organized in thin lobes of highly tracheated tissue that are suspended in the hemolymph. In this way, the fat body has ready access to nutrients, proteins, and hormones that are transported through the hemolymph. The fat body may be present in many different locations within the body cavity; prominent lobes are frequently found in the cavity's center, surrounding the gut. Lobes are also found more closely associated with the epidermis of the insect, somewhat physically separated from the center by the musculature (1). Although this separation of fat body lobes does not necessarily imply that these lobes are structurally or functionally different tissues, the separated tissues are different in a number of species.

The loose structure of the fat body makes it a rather recalcitrant tissue for histological research (21). Proper fixation of the tissues requires special attention (18), and after fixation, it may be difficult to distinguish and separate distinct areas of the fat body. Moreover, the structure of fat body cells causes additional problems. Fat body is normally composed mainly of adipocytes [also called trophocytes (34)]. In addition, the fat body cells are specialized as mycetocytes and urocytes. In cockroaches, mycetocytes host bacterial symbionts (26), and urocytes are degenerate cells that appear to function mostly in the storage and excretion of uric acid (11, 16). The most abundant cell type, the adipocyte, appears to have the broadest range of biochemical reactions. Because these metabolic activities include uptake and storage of nutrients, adipocytes frequently contain large vacuoles; this complicates ultrastructural analysis. Moreover, during development the structure of the fat body changes drastically. In many insects that undergo complete metamorphosis, the fat body

dissociates into single cells before it reaggregates into the adult form, or the tissue is completely histolyzed and the new fat body differentiates from stem cells. As a consequence, defined morphological features are rarely visible during metamorphosis.

Biochemical analyses of fat body tissue are also similarly difficult. It is not always possible to reproducibly select identical regions of the fat body for in vitro experiments. This is especially true during metamorphosis, when cohesive fat body samples cannot be obtained because the dissociated fat body cells are easily disturbed and mixed within the hemolymph. Therefore, few biochemical studies have been conducted on the fat body during this developmental period, and little is known about regional differences or temporal changes in fat body functions. Most information is available on either larval or adult fat body, where the tissue can be obtained as a mass.

STRUCTURAL REGIONALIZATION: DEFINITIONS, LOCATIONS

Although the fat body has been treated mostly as a single tissue, distinct areas have been identified in several species. As mentioned above, in many insects the body musculature separates two fat body regions (1). The area close to the epidermis is called the subcuticular or peripheral fat body, while the central region that surrounds the gut is known as the perivisceral or visceral fat body. Furthermore, clearly distinct lobes of the fat body can be found along the anterior-posterior axis, as reported primarily for dipteran species (67). The different fat body regions are normally only classified according to their location within the body cavity, because no other obvious differences exist. However, in some cases fat body regions can be distinguished visually. Fat body color varies between different insect species; in most cases, the fat body is a white tissue, but it may be yellow, tan, brown, or blue. Color differences within individual insects have been reported in *H. zea* (25) and *P. interpunctella* (71). In these instances, the differences alone did not prove distinct biochemical properties, but they facilitated the segregation of the colored regions during the analysis of the tissue ultrastructure and biochemistry that led to the recognition of different histotypes. In species of Diptera, there are well-documented regional differences in fat body UV fluorescence color (66, 68). As outlined below, fluorescence arises from ommochromes and pteridins that are precursors of eye pigments.

FUNCTIONAL REGIONALIZATION

In Diptera, structural and functional differences between regions of the fat body have long been known. Based on different morphologies, Rizki (67)

recognized six regions of the fat body along the anterior-posterior axis in *Drosophila melanogaster* larvae. Although collectively referred to as the fat body, these regions were subsequently shown to be distinct in their ultrastructure, biochemistry, and gene expression pattern. In most cases, however, protein expression has only been studied in the entire fat body, but not in the separate areas of the tissue. Although some proteins are undoubtedly expressed in all regions, many genes may be expressed exclusively, or at least predominantly, in specific regions of the fat body. For example, the enzymes needed to synthesize ommochromes are produced predominantly in the anterior three segments, close to the final location of the adult eye (67). Differences in the synthesis and uptake of storage proteins have been documented as well. The deposition of storage proteins in protein granules prior to pupation changes the ultrastructure of the fat body drastically. As described in more detail below, the distribution of protein granules within the fat body is not always homogenous. In fact, in some species certain fat body regions do not contain any protein granules. The cytology is also affected because, coincident with the appearance of protein granules in the fat body, other electron-dense structures also form, including autophagic vacuoles (indicative of cell histolysis) and urate granules (for nitrogenous waste). All these ultrastructural differences represent a functional differentiation of the fat body during development.

Regional biochemical differences in other orders have rarely been mentioned. DeLoof & Lagasse (19) reported that peripheral fat body in the Colorado potato beetle, *Leptinotarsa decemlineata,* contains more glycogen than the perivisceral tissue, and therefore may serve as a storage tissue, but no further studies to prove this observation have been conducted. Therefore, the following sections review information pertaining to fat bodies from species of Lepidoptera and Diptera only.

Eye Pigment Deposition

In *D. melanogaster,* differences between the six regions of fat body become visible under UV. Early studies by Rizki (65) demonstrated that kynurenine is solely responsible for the observable fat body autofluorescence at 470 nm when the tissue is excited at a wavelength of 365 nm. Catabolism of tryptophan involves several intermediates, including kynurenine. This product is a precursor for the synthesis of xanthoommatin, a major screening pigment in the compound eye of *D. melanogaster.* Xanthoommatin is not a photoreceptor, but serves as a filter to increase the contrast and reduce the intensity of light of certain wavelengths. In the adult insect, this pigment is concentrated in special pigment granules in the eye. During the larval stadium, its precursor kynurenine accumulates in the fat body, most likely within specialized granules. Kynurenine autofluorescence is confined to the three anterior segments of *D. melanogaster* fat body, probably because the key enzymes for kynurenine

formation are only expressed in these regions. Thus, the kynurenine storage granules reside in proximity to the future adult eye, which forms during metamorphosis. On the other hand, pteridins, the second family of screening pigments, are formed and stored only in the fat body of the posterior three segments (68). These autofluorescent pigments are derived from guanosine nucleotides, and their biosynthetic enzymes are apparently expressed only in the posterior regions of the fat body. The specific autofluorescence of eye pigment precursors thus facilitates the direct visualization of biochemical differences in the various regions of the fat body in these flies.

Urate Storage

Uric acid is the major end-product of nitrogen metabolism in the larval stages of Lepidoptera as it is in other terrestrial insects (16, 18, 34). At the end of the larval period and coincident with the initiation of protein granule formation, the fat body of the skipper butterfly, *Calpodes ethlius,* loses urate oxidase activity and begins producing fibrous granules (53). At the same time, uric acid accumulates in the fat body within the fibrous granules (50). The accumulation of uric acid in the fat body has been reported for several other Lepidoptera, including the silkworm, *Bombyx mori* (74); the tobacco budworm, *Manduca sexta* (6, 91); the giant silkmoth, *Hyalophora cecropia* (32, 75); and the white cabbage butterfly, *Pieris brassica* (45).

During the last larval instar, the frass of *M. sexta* becomes frosted with a white coating (55, 62). The frosting appears at the transition from the feeding stage to the wandering stage and is attributed to a change in uric acid concentration. The appearance of the frosted frass coincides with a rapid decline in the hemolymph levels of uric acid (6). The decline results from a shift in urate metabolism between the Malpighian tubules and the fat body. During this period, the Malpighian tubules cease to remove uric acid from the hemolymph and excrete it in the frass. Concomitantly, the fat body begins the removal of uric acid from the hemolymph and initiates the formation of the uric acid granules within the tissues. The switch from excretion of uric acid to storage in the fat body is correlated with the peak of ecdysteroids that initiates the wandering stage and the onset of metamorphosis (4) and is stimulated by 20-hydroxyecdysone and inhibited by juvenile hormone (5).

The uric acid storage occurs in discrete membrane-bound vacuoles that have an electron-dense fibrous matrix (7). These storage vacuoles are initially concentrated around the nucleus, and they are present in all of the fat body cells. During the larval-pupal transformation, the urate storage vacuoles double in size and become uniformly distributed throughout the cytoplasm (7). Within the vacuoles, the uric acid forms fibers that are individually surrounded by proteins (8). The vacuoles contain 75–78% uric acid, and the proteins present in the vacuoles range in molecular weight from 20,000 to greater than 200,000.

Associated with the urate storage vacuoles, but not the urate fibers, is the enzyme xanthine dehydrogenase (9), which is responsible for catalyzing the synthesis of uric acid in the uricotelic pathway (16). The xanthine dehydrogenase was localized immunocytologically within the storage vacuoles and near the basal lamina of the fat body cells (9). During the last larval instar, the overall urate synthesizing capacity decreases (91), as does the xanthine dehydrogenase activity (9).

The urate storage vacuoles remain within the fat body cells until the later period of pharate adult development (22). Following the decline in ecdysteroid titers during this phase, the uric acid is mobilized from the storage vacuoles. Treatment of the pharate adults with 20-hydroxyecdysone 13–15 days after pupation inhibits the release of the uric acid into the hemolymph, but treatment has no effect after this period. The dissolution of the urate from the storage vacuoles may involve lysosomal activities. Although urate storage vacuoles are reportedly present within all fat body cells (7), only the perivisceral fat body was examined (JS Buckner, personal communication). Because there is a strong likelihood of more than one fat body histotype in larvae of *M. sexta,* as found in *H. zea* and *P. interpunctella,* that were neither recognized nor tested, it would be reasonable to reexamine the major shifts in urate synthesizing capacity in the context of changes in synthetic activities between (or among) multiple fat body histotypes. Further consideration of this possibility should be included in future examinations of the urate excretion-storage transition.

Storage Proteins in Diptera

SYNTHESIS AND UPTAKE Although storage proteins are synthesized throughout the whole larval stadium, the major period of maximal expression occurs during the last larval instar (47). The proteins are released into the hemolymph, where they reach concentrations up to 60 mg/ml. Once feeding ceases and pupariation begins, storage proteins are no longer expressed. Instead, the proteins that have accumulated in the hemolymph are removed and taken up into fat body tissue, where they are incorporated into protein granules. The uptake is selective: storage proteins are sequestered rapidly and efficiently, while only minor amounts of other hemolymph proteins accumulate in the fat body (36, 54, 79). In *D. melanogaster,* storage proteins are sequestered preferentially by the posterior regions of the larval fat body. Tysell & Butterworth (78) found relatively few protein granules in the anterior three segments, but abundant numbers in the posterior regions. Later, Butterworth & Rasch (14) suggested that this region-specific accumulation may be the result of differential expression of a yet unidentified storage protein receptor in the posterior regions.

The site of synthesis of fat body storage proteins is not known; they may be produced in a tissue different from the the tissue of eventual sequestration. Thus, storage proteins may be produced predominantly in the anterior segments of the fat body but accumulate in the posterior segments. A differentiation of the fat body into two separate tissues, with respect to storage protein metabolism, would be logical from a physiological point of view. Segregating function to histotype would solve the enigma of why these proteins are first synthesized and then released into the hemolymph, only to be taken up by the fat body soon afterwards. However, some data suggest that two storage proteins of *D. melanogaster,* LSP-1 and LSP-2, are expressed at similar levels in all six of the fat body regions (31, 56, 69). Unequivocal evidence of regional synthesis and sequestration of storage proteins in the fat body requires additional examination of the cytolocalization of storage protein expression as well as of their receptors within different fat body regions using newly developed immuno and molecular probes.

STORAGE PROTEIN RECEPTORS Although none have been found in *D melanogaster,* storage protein receptors occur in other species of Diptera. Ueno and coworkers (79, 81) identified a storage protein receptor from the fat body of the flesh fly *Sarcophaga bullata.* Although the protein had not been purified to homogeneity, in vitro binding and ligand blot assays demonstrated that the receptor protein is an integral membrane protein with a molecular weight of 120,000. The receptor protein apparently binds the storage protein with a binding constant of 4×10^9. Interestingly, the protein is synthesized as a cryptic, inactive precursor that is converted to the biologically active receptor by proteolytic cleavage of a 5000-Dalton peptide (80). Similar experiments in another fly species, *Calliphora erythrocephala,* revealed a more complex picture (10). In ligand blots at least three protein bands interacted with the main storage protein, calliphorin. However, whether these proteins are independent receptors or are all members of a complex, multimeric receptor family remains to be resolved. Clearly, the limited information available on storage protein receptors in Diptera makes the study of regional differences difficult. To analyze whether the receptor is indeed located preferentially in the posterior regions of the fat body (14), investigators will need antibodies to establish the cytolocalization and distribution of the receptor(s) within the tissue regions. Furthermore, the temporal and spatial patterns of receptor expression could also be followed using in situ hybridization, but as sequence information for the receptor is lacking, oligonucleotide probes are not available for these experiments or for cloning the receptor cDNA. Thus, further progress depends on the purification of sufficient quantities of homogenous receptor protein. This represents a challenging task, because the large amounts of fat body necessary for developing purification procedures for integral membrane proteins are difficult to prepare from a small insect such as *D. melanogaster.*

These studies demonstrate that fat body tissue in *D. melanogaster,* and other species of Diptera, is heterogeneous along the anterior-posterior axis and that the posterior areas are more involved in the storage of proteins. However, not all species of Diptera manifest this organization. The midge *Chironomus thummi* exhibits specialization of fat body tissues between the subepidermal (peripheral) and perivisceral fat body. Larvae of this insect use hemoglobin as their main storage protein (70). Vafopoulou-Mandalos et al (82) demonstrated with radiolabeled precursors of the chromophore that hemoglobin is synthesized exclusively in the peripheral fat body, from which it is released into the hemolymph. Thus, the *C. thummi* fat body has also differentiated into two distinct fat bodies in which the specific functions of hemoglobin synthesis and metabolite storage are segregated between the two regions.

Storage Proteins in Lepidoptera

SYNTHESIS AND UPTAKE The physiological processes of storage protein metabolism in Lepidoptera are fairly well understood. All species of Lepidoptera manifest storage proteins, and it has been shown that these proteins are made predominantly, but not exclusively in the fat body (33, 73). Following their release into the hemolymph during the ultimate larval stage, the storage proteins are taken up by the fat body shortly before pupation and deposited in protein granules within the fat body.

The biosynthesis of storage proteins has been studied in several species, including *B. mori* (76, 77); *C. ethlius* (57); the greater wax moth, *Galleria mellonella* (61); *M. sexta* (15, 64); and the imported cabbage moth, *Pieris rapae* (35). In all cases, the proteins are synthesized in early larval stages. The amount of arylphorin, a storage protein, in the hemolymph fluctuates throughout development but reaches its maximum during the ultimate larval instar. During the prepupal period, all storage proteins are taken up selectively by fat body tissue, albeit to varying degrees (73).

Shortly before pupation, storage protein synthesis is terminated, most likely at the transcriptional level, as demonstrated for two storage proteins from *M. sexta* (90). Ecdysone causes the termination of arylphorin expression. Although storage protein synthesis has been studied several times in dissected fat body in vitro, few attempts have been made to measure the biosynthetic capacity of different regions of the fat body. However, in *H. zea* and *P. interpunctella,* species of Lepidoptera that exhibit visible regional differences, only certain areas of the fat body participate in storage-protein synthesis (25, 71). Though limited, these observations nevertheless raise the prospect that only certain areas of the fat body are active in the expression of storage proteins.

Studies on aspects of storage protein uptake and metabolism in the fat body have been conducted in several of the same and other insects, including *B. mori* (77), *C. ethlius* (18), *H. zea* (85), *H. cecropia* (75), *M. sexta* (38), *P. rapae* (35), and *P. interpunctella* (71). The most detailed ultrastructural studies of the entire process of protein uptake, storage, and utilization have been done on *C. ethlius*. Similar processes appear to occur in other species of Lepidoptera.

Calpodes ethlius The ground-breaking ultrastructural studies on *C. ethlius* in Michael Locke's laboratory showed that storage protein uptake into fat body occurs via endocytosis. Using horseradish peroxidase as a model protein, this group demonstrated the individual stages of endocytosis, from the initial formation of an endocytotic vesicle to the final protein granule (51, 52). The data suggest that early endocytotic vesicles contain sequestered proteins and fuse within the fat body to form multivesicular bodies. Later, the vesicular bodies are attacked by primary lysosomes, and hydrolytic enzymes then digest membrane components as well as nonspecifically sequestered hemolymph proteins. Ultimately, the storage proteins begin to crystallize within the resulting granules. However, the nonspecific sequestering of a marker enzyme not normally present in the hemolymph has led others to question the strict specificity of storage protein uptake (73). Because storage proteins account quantitatively for the majority of hemolymph proteins, the storage proteins should be expected to be taken up as the predominant component even in a nonspecific pinocytotic process. On the other hand, Pan & Telfer recently demonstrated that the fat body takes up storage hexamers to a much larger extent than injected foreign proteins, probably by means of receptor mediated endocytosis (58).

In none of these studies was the attempt made to identify the regions of fat body that synthesize or sequester the proteins. To explain the fact that storage proteins are synthesized in the fat body early in the last larval stage, but taken up again by the tissue towards the end of this stage, Dean et al (18) proposed that the tissue switches from synthesis to storage prior to pupation. However, in *P. interpunctella* and *H. zea,* in which segregation of histotypes has been observed, different fat body regions are responsible for storage protein synthesis and uptake (25, 71).

Plodia interpunctella Two different fat body histotypes that are regionally segregated within the hemocoel have been described for the larvae of *P. interpunctella* (71). The histotypes are segregated along the anterior-posterior axis with the tan-colored fat body localized posteriorly and the white-colored fat body localized anteriorly. The tan fat body is highly tracheated, has numerous large mitochondria, and is the predominant form in the first three instars. It undergoes histolysis at pupation, and is therefore restricted to the larval stage. It can synthesize and secrete storage proteins but cannot accumu-

late them in storage granules. Only the white fat body forms storage protein granules in pharate pupae and undergoes reorganization to give rise to the adult fat body during metamorphosis.

Tan fat body has been found in other members of the pyralid subfamily Phycitinae, namely the navel orange worm, *Amyelois transitella,* and the almond moth, *Cadra cautella,* and shares all of the characteristics of tan fat body found in *P. interpunctella* (PD Shirk & G Zimowska, unpublished data). In the more distantly related pyralid moth *G. mellonella* (subfamily Galleriinae), tan fat body was also found in the earliest instars, but not in the last two instars (PD Shirk & G Zimowska, unpublished data). Tan fat body was not found in members of two other pyralid subfamilies: the meal moth, *Pyralis farinalis,* and the pickleworm, *Diaphania hyalinata.*

The presence of the tan fat body may provide the members of phyticine pyralids with the ability to produce metabolic water. Many of these moths are pests of stored products and feed on grains with low moisture content. The presence of the tan fat body with its numerous, large mitochondria would provide a resource for the production of metabolic water that would permit these insects to feed on these grains. The last instar of *G. mellonella* produced metabolic water when maintained in a low humidity environment (27). This study also revealed a high degree of uncoupling of mitochondrial respiration from oxidative phosphorylation in the larval fat bodies of *G. mellonella* (54.4–59.6%) and the Mediterranean flour moth, *Anagasta kühniella* (52%). Although the report did not indicate the type of fat body used in the measurements, the results suggest that if uncoupling occurs to an equivalent level in the tan fat body of these moths, they should be able to produce considerable amounts of metabolic water.

Helicoverpa zea A coherent picture has developed from the recent work on fat body tissues of *H. zea.* As mentioned above, two distinct fat body regions are easily identified in prepupae of this insect. The perivisceral fat body located in the body cavity, between the gut and the outer muscle layer, is colored blue, while the white peripheral fat body resides as a thin layer between the muscles and the integument (25). The distribution is also visible in cross-sections of larvae embedded in paraffin and stained with eosine and hematoxylin. As shown in Figure 1, perivisceral fat body appears more intensely stained because of its higher protein content. The color differences facilitated the separation of the tissues and the analysis of their roles in storage protein synthesis and uptake. In early larval stages, only the peripheral fat body is visible, and little if any fat body tissue can be found in the body cavity. In the last larval instar, the perivisceral fat body appears. At first, it is not colored, but early in the instar the blue color becomes clearly visible. The blue tissue becomes the dominant fat body during the last 4 days of the ultimate larval instar (85).

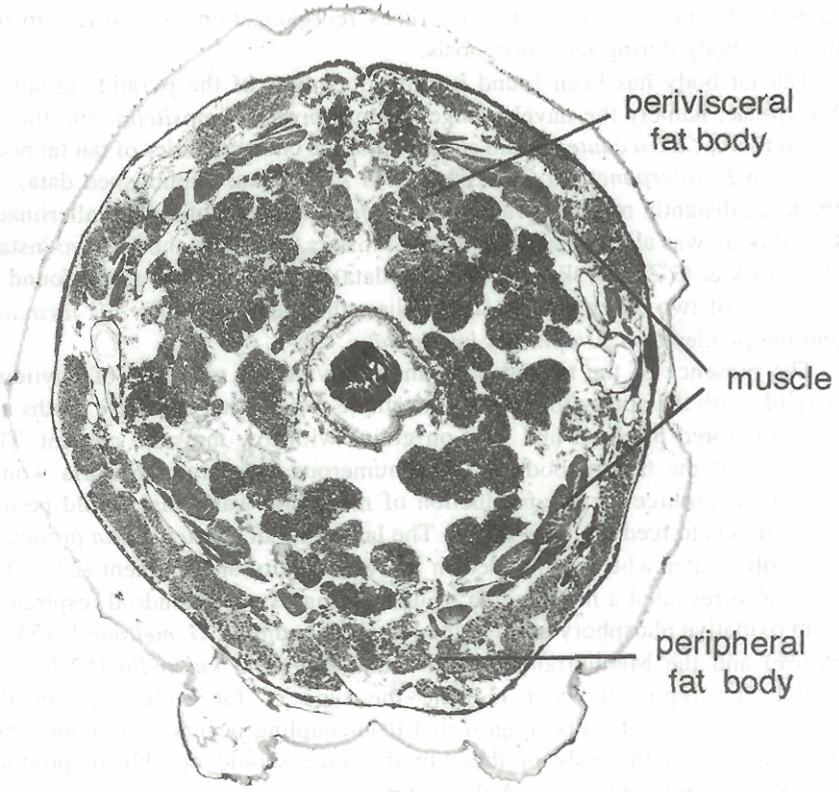

Figure 1 Location of perivisceral and peripheral fat body in *Helicoverpa zea*. Prepupae were fixed with glutaraldehyde and embedded in paraffin. Sections were stained with eosine/hematoxylin (88). Perivisceral fat body is stained more intensely because of its higher protein content.

In vitro incubation of fat body tissue with radiolabeled amino acids showed that the peripheral fat body synthesizes many proteins, including storage proteins. Their synthesis ceases during day 5 of the last instar. The blue perivisceral fat body, however, never secretes any radiolabeled proteins into the surrounding medium, indicating that this tissue is not capable of secreting storage proteins (25).

The functional roles are reversed for protein uptake and storage. Peripheral fat body tissue was never found to contain any amount of sequestered storage protein, as determined by injecting radioiodinated storage proteins. However, perivisceral fat body rapidly sequestered these proteins. In fact, within the three-day period immediately before pupation, the hemolymph concentration

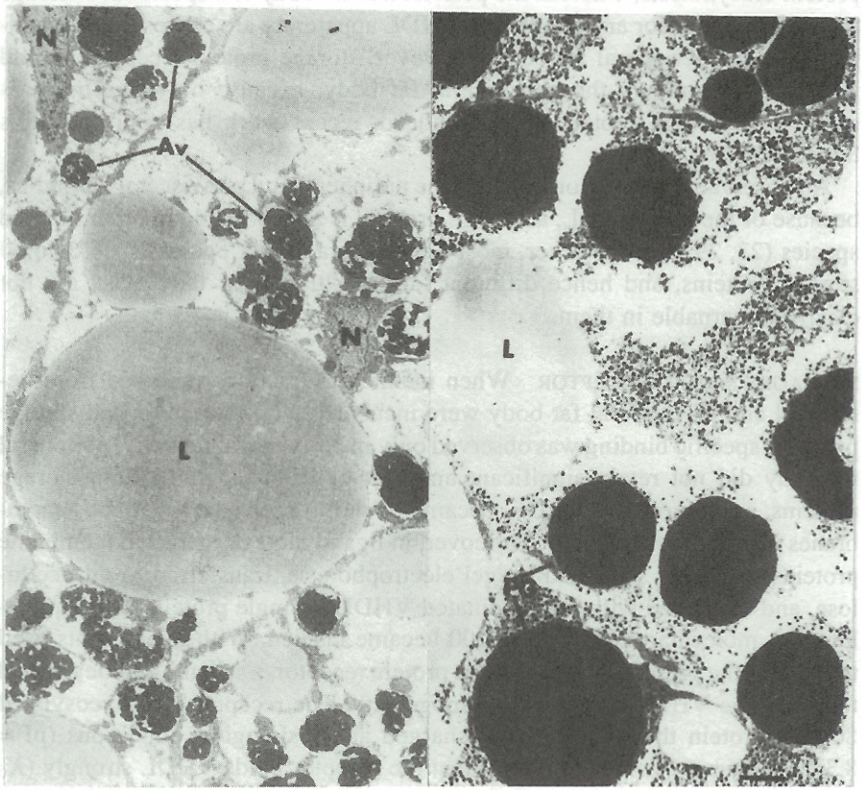

Figure 2 Electron micrographs of peripheral and perivisceral fat body of *Helicoverpa zea* prepupae. Tissue was dissected, fixed, and processed as previously described (87). (*Left panel*) Peripheral fat body; (*right panel*) perivisceral fat body. Abbreviations: AV, autophagic vacuole; PG, protein granule; L, lipid droplet; N, nucleus. Size bar = 1 μm.

of arylphorin and very-high-density lipoprotein (VHDL), the known storage proteins of *H. zea,* diminished from 60 and 25 mg/ml, respectively, to concentrations less than 10 and 1 mg/ml. At the same time, both proteins accumulated in the perivisceral fat body. Thus, the blue color of the perivisceral fat body originated from sequestered VHDL that is colored intensely blue because of noncovalently bound biliverdin. The selective uptake of storage proteins into perivisceral fat body is also visible in electron micrographs. At pupation, protein granules are abundant in perivisceral fat body, while autophagic vacuoles are prominent in the peripheral tissue (85) (Figure 2).

The above discussion demonstrates a clear distinction between the fat body regions in *H. zea.* The peripheral fat body is mainly responsible for larval

protein biosynthesis, whereas the perivisceral fat body is a specialized storage organ. The genes for arylphorin and VHDL apparently are expressed predominantly in the peripheral fat body. However, storage protein receptors should be expressed only in the perivisceral fat body, because only this tissue is capable of storage protein uptake. Indeed, recent work has confirmed this hypothesis (see below).

An observable distinction between the peripheral and perivisceral fat bodies, because of the blue VHDL, a storage protein, is also possible in other noctuid species (23, 29, 60). However, most Lepidoptera do not possess such colored storage proteins, and hence different fat body tissues, if they exist, are not clearly discernable in them.

STORAGE PROTEIN RECEPTOR When membrane fractions prepared from peripheral and perivisceral fat body were incubated with radioiodinated storage proteins, specific binding was observed only in perivisceral fat body; peripheral fat body did not retain significant amounts of label (87, 88). Both storage proteins, arylphorin and VHDL, became bound to perivisceral fat body membranes in a saturable manner. Moreover, in ligand blots of extracted membrane proteins that were separated by gel electrophoresis, transferred to nitrocellulose, and incubated with radioiodinated VHDL, a single protein band equivalent to a molecular weight of 80,000 became labeled. With ligand blots used to monitor the presence of the storage protein receptor, a scheme was developed to purify the VHDL receptor to homogeneity. The receptor is a glycosylated 80-kDa protein that is negatively charged at physiological conditions (pI = 8.3). Binding experiments suggest that the receptor binds VHDL strongly ($K_d = 7.8 \times 10^{-8}$); the binding is Ca^{2+} dependent, as has been reported for many other receptor proteins. The pH optimum for the binding is approximately 7.5, which corresponds to the pH of the hemolymph when VHDL is taken up by the fat body (87).

When similar experiments were performed to isolate the arylphorin receptor, the results were almost identical. Membranes bind arylphorin in a saturable manner, with a binding constant of 8×10^{-8}, at a pH optimum of 7.5; Ca^{2+} is also required (88). As shown by ligand blots, the receptors for both storage proteins have identical physical properties, suggesting that they might share a single receptor. Indeed, this result was confirmed with competition experiments: an excess of unlabeled arylphorin prevented the binding of radiolabeled VHDL to the receptor in the same manner as an excess of unlabeled VHDL reduced arylphorin binding. Other proteins had no influence on the binding of either storage protein. Therefore, the investigators concluded that in *H. zea* both major storage proteins share a single receptor despite their pronounced structural differences. Further research is needed to identify the structural motifs of the storage proteins that are recognized by the receptor.

With immuno-gold labeling techniques, the storage protein receptor is detectable only in perivisceral fat body, localized primarily on the cell membrane (87). Therefore, the receptor probably mediates the uptake of the storage proteins into the perivisceral fat body. Receptor-mediated endocytosis of the storage proteins was demonstrated subsequently with gold-labeled storage proteins (89). When the perivisceral fat body was incubated for varying time intervals with storage proteins that were labeled with 5-nm or 15-nm gold particles, all the intermediate cytoplasmic structures expected in receptor-mediated endocytosis contained gold particles. Initially, coated pits formed at the membrane that budded off to form coated vesicles. Subsequently, endosomes that had lost their protein coat fused to form multivesicular bodies that eventually gave rise to protein granules. Because the storage protein receptor was detectable with specific antibodies in all of these structures as well, the receptor itself is probably also incorporated into the protein granules and not recycled.

Initially, protein granules contain both arylphorin and VHDL, as expected from a shared receptor. Within 4 days after pupation, however, VHDL disappears completely from the granules, which at the same time become crystalline (85). Apparently, membrane components, VHDL, and the storage protein receptor are readily degraded in the initial protein granule, while arylphorin, in its crystalline state, resists proteolytic attack. Arylphorin containing granules are partially digested in the latter half of the pupal stage, presumably to supply amino acids for the synthesis of adult structures. Many granules, however, persist into the adult stadium.

DEVELOPMENTAL DIFFERENCES

As the main biosynthetic and storage tissue of insects, the fat body is of crucial importance in all stages of the insect's life. During metamorphosis of holometabolous insects, virtually all organs and tissues change and adult-specific proteins are expressed, while production of larval- and pupal-specific proteins is terminated. Thus, it is not surprising that the fat body cells as well as the tissue itself undergo a radical exchange of cytoplasmic inclusions and gross structure to accommodate the newly required functions. These changes are usually immediately visible upon observation of the gross anatomy: larval tissue is frequently arranged in sheets, whereas the adult fat body has nodular clusters. Differences are also obvious in the cellular ultrastructure (18), e.g. the relative amount and shape of vacuoles, mitochondria, and endoplasmic reticulum are altered. Particularly obvious in species of Lepidoptera and Diptera is the appearance of electron-dense structures in the fat body during the prepupal period. These structures include autophagic vacuoles (which suggests fat body histolysis), protein granules (mainly from storage protein uptake), and urate granules (from nitrogenous waste). Protein granules, in particular, are

absent in early larvae but become prominent in the fat body of pupae and adults. Thus, the larval and adult fat body typically show few similarities. The differences between the larval and adult fat body are often so dramatic that it is not easy to decide whether the fat body in each of these developmental stages is derived from the same tissue.

Two fundamentally different mechanisms have been suggested to explain these changes: (*a*) the complete destruction of larval fat body and simultaneous synthesis of adult fat body from undifferentiated stem cells, and (*b*) a process called cell remodeling (46) in which larval fat body tissue is dissociated to isolated cells that reassociate to form the adult fat body. Apparently, both mechanisms are used to a varying extent in different insect orders. Most of the evidence published for Diptera suggests that the adult fat body is newly differentiated tissue, and the larval fat body is histolyzed. In contrast, studies in *C. ethlius* indicate that in Lepidoptera the larval fat body is not eliminated; instead, the tissue is broken down into single cells that reassociate to form the adult fat body (18). For most insect species, however, little is known about the origin of the adult fat body and the fate of the larval fat body, other than the fact that the gross morphology of both tissues frequently differs. The dramatic changes in morphology and tissue organization that occur during metamorphosis make studying fat bodies in the pupal stage by biochemical or ultrastructural methods very difficult, and thus only a few authors have attempted to follow the fat body through the entire pupal stadium.

Cell Death During Metamorphosis

At metamorphosis, the larval fat body of holometabolous insects dissociates from a cohesive tissue into individual cells free within the hemocoel (18). A series of elegant experiments conducted by Kurata, Natori and colleagues (37, 40–43) have demonstrated the function of the hemocytes in the dissociation of the larval fat body. This group has shown that during metamorphosis, the dissociation of the larval fat body in the flesh fly *Sarcophaga peregrina* is moderated by the activity of pupal hemocytes and depends on two pupal-specific proteins in the hemocytes: (*a*) a cell-surface protein that is central to the recognition of fat body basement membrane and (*b*) a cysteine proteinase that digests the basement membrane to effect dissociation. After pupariation, the hemocytes produce new proteins that are incorporated into the plasmalemma and appear as cell-surface proteins. These proteins included a 200-kDa protein essential for hemocyte recognition of the basement membrane of the fat body (41). The central role in hemocyte fat body recognition was demonstrated with antibodies to the protein; binding interfered with the 200-kDa protein and thus inhibited the dissociation of the fat body (40, 41). The dissociation is also competitively inhibited by the addition of the purified 200-kDa protein to the dissociation medium (37). In addition, the purified protein binds specifically

with the basement membrane of the fat body in a saturable manner (37). After puparium formation, the number of hemocytes expressing the 200-kDa protein increases significantly and in one experiment reached a maximum about 10 h after pupariation (40). These hemocytes are anucleate, as indicated by an inability to stain with DAPI (diamidinophenylindole), and they associate with the fat body cells.

After the pupal hemocytes bind with the basement membrane, a 29-kDa hemocyte proteinase is released that digests the membrane and effects the dissociation of the fat body (40, 42, 43). Incubation of pupal hemocytes with a triple combination of monoclonal antibodies to the 200-kDa protein induced the release of the proteinase activity (40). The enzyme was shown to be a cysteine proteinase, although it shared substrate specificity for synthetic substrates of serine proteases such as chymotrypsin and cathepsin B (42). The purified 29-kDa cysteine proteinase is sufficient to dissociate the larval fat body, and antibodies to the proteinase inhibited the dissociation (43). The amount of the proteinase increases in the same time course as the 200-kDa cell surface protein. The 29-kDa cysteine proteinase is localized in granules within anucleate hemocytes similar in morphology to those that express the 200-kDa protein.

Following dissociation during pupariation, the larval fat body persists as independent cells within the hemocoel until the adult stage in *D. melanogaster* (67). During the larval stage, the cell number remains constant at about 2200 larval fat body cells (13, 67). Following the initiation of metamorphosis, the number of larval fat body cells begins to decrease gradually until 3 days after adult eclosion when no more larval fat body cells can be found (13). Histolysis is the likely cause of this decrease (12).

With regard to the question of whether histolysis or remodeling of the larval fat body cells occurs (18), evidence from several recent studies indicates that these cells actually undergo histolysis after initiation of metamorphosis. As the larval fat body cells progress through metamorphosis, their size decreases owing to the loss of reserve substances such as lipid droplets, glycogen deposits, and protein granules (13). In addition, the size of the nuclei and the amount of DNA decrease, and autophagosomes appear that contain remnants of organelles during the later pupal stages. If remodeling of the larval fat body was occurring, the number of adult fat body cells should increase. However, the number of adult fat body cells in females remains constant at about 18,000 cells after adult eclosion (28). The use of trypan blue revealed the presence of dead larval fat body cells after adult eclosion (63). Larval fat body cells removed 4 h after eclosion from adult females of the Canton-S strain of *D. melanogaster* and cultured all stained with trypan blue within 24 h, indicating that these cells had died. This was not the case for larval fat body cells taken from postfeeding third-instar larvae, pupae at head emersion, or preemergent

adult females. Taken together, these data indicate that cell death and histolysis is the mechanism for removal of the dipteran larval fat body.

Using the *ap4* mutant that is deficient in juvenile hormone, Postlethwait & Jones (59) found that juvenile hormone regulated the histolysis of the larval fat body. However, the amount of juvenile hormone in the flies decreases during the first day after adult eclosion (3). This observation is contrary to the hypothesis of juvenile hormone–stimulated histolysis. The role of juvenile hormone was reexamined using other alleles of the *apterous* gene that also resulted in low juvenile hormone production by adult female corpora allata (63). Only the larval fat body of *ap4* flies responded to juvenile hormone treatment by undergoing histolysis. The larval fat body of the wild-type and other alleles progressed through a normal loss of cells regardless of the juvenile hormone levels. These findings suggest that larval fat body histolysis is not a juvenile hormone–mediated process but is regulated otherwise.

However, some evidence indicates that the larval fat body survives for extended periods in the adult stage. The anterior larval fat body cells of the *rc* mutant can be induced to produce a red pigmentation that was used as a cell marker to follow the fate of the anterior fat body cells (67). The red *rc* fat body cells were found primarily in the head capsule and thorax of the pupae and adults, suggesting that some of these cells survived after adult eclosion. Larval fat body cells are functionally competent in the adult stage. Treatment of isolated adult abdomens with 20-hydroxyecdysone stimulated the synthesis of a larval serum protein, LSP-2, that is normally only produced by the larval fat body (30). Addition of methoprene, a juvenile hormone analogue, to the treatment regime inhibited the 20-hydroxyecdysone–stimulated synthesis of LSP-2. The authors attributed the inhibition to juvenile hormone induction of larval fat body histolysis but did not confirm this hypothesis. However, new synthesis of radiolabeled LSP-2 and the transcript for LSP-2 were detectable in normal 4- to 8-day-old adults (72). In Western blots probed with LSP-2 antibodies, significant amounts of LSP-2 were detected in the hemolymph of flies 0 to 21 days old (2). In situ hybridization localized the LSP-2 transcript to the fat body contained within the head capsule of the adult flies. Quantitative transcript analysis showed that over 80% of the LSP-2 transcript was present in the head capsule with 15% in the thorax and 5% in the abdomen. Considering the results from the *rc* mutant, the expression of LSP-2 in the adult may result from surviving larval fat body. This possibility could probably be resolved by transplantation of a transgenic fly's larval fat body with an easily identifiable marker into a neutral host fly.

Cell Remodeling During Metamorphosis

Unlike in the cyclorrhaphous Diptera, the major mechanism of generating the adult fat body in the Lepidoptera is believed to involve remodeling of the larval

fat body, i.e. the destruction and replacement of cell structures as function changes during development (18, 46). The process of eliminating the cytoplasmic inclusions and subsequently replacing them with the new organelles required for the new functional activities of the fat body has been reviewed (18). Fat body remodeling has been extensively examined in *C. ethlius* (18) and has been confirmed in the other species of Lepidoptera that have been examined: *B. mori* (77); *A. kühniella* (17); *G. mellonella* (21); *H. cecropia* (75); *H. zea* (86), the cynthia moth, *Phylosamia cynthia ricini* (83); and *P. rapae* (35).

In Lepidoptera, the general course of changes in the fat body begins during the last larval instar, as reviewed by Dean et al (18). The larval cytoplasmic inclusions involved in the synthetic process, such as the peroxisomes, microbodies, mitochondria, and rough endoplasmic reticulum (RER), are consumed in autophagic vacuoles. At the same time, storage organelles, such as lipid vacuoles, glycogen granules, multivesicular bodies, and storage protein granules, appear in the cytoplasm. As in the Diptera, the fat body dissociates at the larval-pupal transformation, and the dissociation is most likely facilitated by the action of the hemocytes as well.

The fat body cells of Lepidoptera maintain the same morphology until after they reassociate. The reassociation of the cells to form the adult fat body requires the activity of hemocytes, as does the dissociation (84). The nonmotile dissociated fat body cells of the polyphemus moth, *Antheraea polyphemus,* became reaggregated when the ameboid hemocytes moved them together. The reaggregation was blocked by the elimination of the ameboid hemocytes or by the addition of ethylenediaminetetraacetic acid (EDTA) to chelate the divalent ions in the culture medium. The transplantation of thymidine-labeled larval fat body cells into a nonlabeled larval host established that larval fat body can undergo remodeling to produce the adult fat body in Lepidoptera (39). Intact sections of thymidine-labeled adult fat body from *H. cecropia* were found in the adult host after metamorphosis, demonstrating that not only did the cells of the larval fat body undergo remodeling but that they maintained their general associations with neighboring fat body cells during the period of dissociation. Following the reassociation of the fat body, the mitochondria, RER, and Golgi apparatus were again produced to carry out the synthetic functions of the adult fat body (18).

Selective Cell Death and Remodeling

In the cases described above, fat body differences were examined only from a temporal, but not a spatial point of view. Given the two distinct tissues in *H. zea* and *P. interpunctella* larvae, a separate consideration of the developmental fate of each fat body seems appropriate. In a detailed ultrastructural analysis, the peripheral fat body of *H. zea* was shown to be histolyzed during the pupal stadium (86). As the peripheral fat body functions throughout the

whole larval stadium as a biosynthetic organ, it is rich in mitochondria and endoplasmic reticulum. Shortly before pupation, however, its biosynthetic capacity declines. Mitochondria and RER are reduced, while autophagic vacuoles become more abundant. In early pupae, mitochondria disappear almost completely, and the plasma membrane is digested in many cells. During the subsequent days, most lipid droplets, many autophagic vacuoles, and large parts of the plasma membrane disappear. Simultaneously, the peripheral fat body loses integrity and ceases to be a distinct tissue. The remaining structure represents fragments of cells, and after adult eclosion, cells from the peripheral fat body are not detectable.

The perivisceral fat body, on the other hand, is first distinguishable at the end of the penultimate larval instar, but its structure is similar to that of the peripheral fat body. In contrast to this tissue, the mitochondrial and RER content of the perivisceral fat body increases during the last larval instar. The most obvious distinction between the peripheral and perivisceral fat bodies is the uptake of storage proteins, which begins in the perivisceral fat body at day 5 of the last instar. The overall structure of this tissue does not change until after the initiation of adult development when the protein granules are hydrolyzed and the synthetic organelles reappear, indicating that the perivisceral fat body has assumed major biosynthetic tasks. This tissue is the only fat body present in adults. Thus, the adult fat body in *H. zea* is clearly derived from the perivisceral larval fat body tissue, although the adult organ is spread throughout the entire body cavity (86).

Similar observations have been made in *P. interpunctella* (PD Shirk, unpublished results): The tan fat body is histolyzed during the pupal stage, while the white fat body undergoes cell remodeling. As in *H. zea,* the histotype that predominates during the early larval instars and does not accumulate storage products at the larval-pupal transformation is histolyzed in the last larval instar. Only the storage fat body from these insects was remodeled and survived into the adult stage. These studies indicate that the functional activities of the larval and adult fat bodies of these two insects are segregated into two developmentally restricted histotypes similar to that observed in the cyclorrhaphous Diptera. Clearly, in these two species of Lepidoptera cell death as well as cellular remodeling play important roles in the transformation of the larval fat body to the adult tissue.

Other Orders

As mentioned above, relatively little is known about the development of fat body in other insect orders. However, some interesting data exist for *L. decemlineata* (20, 44). Histological evidence indicates that the peripheral fat body of beetle larvae is completely histolyzed during metamorphosis, whereas the dorsolateral fat body gives rise to the adult fat body. Although nothing is known about different biochemical properties of these tissues, the findings may indi-

cate that regional differentiation into two distinct larval fat body tissues may be common in insects.

CONCLUSIONS AND FUTURE DIRECTIONS

Links between structural and functional activities for various regions of the fat body from numerous insects have been established. Despite this evidence, no unifying concept has emerged to predict a correlation between the functional activities and pleomorphic histotypes of the fat body. However, treatment of the fat body as a homogenous tissue overlooks the obvious conclusion that this organ is more than a simple structure.

So far, only those histotypes identified by location, color, fluorescence, or ultrastructural appearance have been investigated. These criteria cannot be universally applied to insects and thus offer only limited utility for discerning fat body histotypes. How then should the identification of various fat body histotypes be approached? Rather than relying on gross or ultrastructural differences, molecular markers, e.g. antibodies or DNA sequences that reflect functional activities will most likely provide the tools necessary to distinguish various histotypes. For example, storage protein receptors are good candidates for analyzing the functional regionalization of biosynthetic and storage fat bodies in Lepidoptera and Diptera, because the receptors are present exclusively in cells that sequester storage proteins from the hemolymph. However, the utility of these markers will be limited until receptors from other species are identified and characterized.

The regional and developmental differences described in this paper have convinced us that they represent only a minor fraction of the specialized differentiation of fat body histotypes that exists in insects. Because of the rapid improvement of sensitive biochemical, immunological, and molecular techniques, many more regional differences in protein content and selective gene expression will undoubtedly be discovered. These regional and functional differences in the fat body tissue will most likely represent altered developmental paths for portions of this organ that provide specific beneficial adaptations for each species.

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