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Plasma membranes from insect midgut cells

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ABSTRACT

Plasma membranes from insect midgut cells are separated into apical and basolateral domains. The apical domain is usually modified into microvilli with a molecular structure similar to other animals. Nevertheless, the microvillar structure should differ in some insects to permit the traffic inside them of secretory vesicles that may budd laterally or pinch-off from the tips of microvilli. Other microvillar modifications are associated with proton-pumping or with the interplay with an ensheathing lipid membrane (the perimicrovilllar membrane) observed in the midgut cells of hemipterans (aphids and bugs). The perimicrovillar membranes are thought to be involved in amino acid absorption from diluted diets. The microvillar and perimicrovillar membranes have densities (and protein content) that depend on the insect taxon. The role played by the microvillar and perimicrovillar proteins in insect midgut physiology is reviewed here trying to provide a coherent picture of data and highlighting further research areas.

Key words: microvillar membranes, perimicrovillar membranes, nutrient absorption, ion transport, water transport, midgut molecular physiology.

INTRODUCTION

Midgut cells are associated one another by junctions that separate the plasma cell membranes into an apical and a basolateral domain. The apical domain is usually modified into finger-like projections, the microvilli, whose shape are ensured by a core of actin that are held in place by various ancillary proteins (see below). The insect microvilli may be modified by the presence of inner mitochondria or an ensheathing membrane (the perimicrovillar membrane) or to make possible unique secretory mechanisms. The basolateral domain has peculiar intercellular junctions in its lateral part (Lane et al. 1996), whereas the basal part may be modified into varied infoldings.

It is known since a long time that insect midgut cell apexes are involved in the transport of water (Wigglesworth 1933) and organic compounds (Treherne 1959). Nevertheless, only after 1980 it was recognized that insect midgut apical cell membranes play a role in digestive events. Before 1980, all insect digestive enzymes were considered to be secreted like among mammals till 1961. In mammals, Miller and Crane (1961) provided cell fractionation data showing that disaccharidases are firmly bound to the cell membrane covering the entero-

Dedicated to the memory of Prof. Francisco Lara (1925–2004).

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cyte microvilli.

Ferreira and Terra (1980) succeeded in isolating microvilli from an insect midgut having a single cell type (midgut caeca from a lower Diptera, that is a sciarid fly) by using a differential calcium precipitation technique (Schmitz et al. 1973) developed for mammals. The technique consists in homogenizing the tissue in a small Waring blendor, followed by the addition of a divalent cation (usually Ca^{++}). Calcium ions causes agglutination of cell membranes, except microvillar ones, because of the electronic charge associated with their glycocalyx. The supernatant of low speed centrifugation is enriched with microvilli (electron microscope controls) that are collected by medium speed centrifugation. Similar results were obtained by time-consuming differential centrifugation. A few months later, Hanozet et al. (1980) attempted to isolate microvilli from the columnar (principal) cells of a tissue (composed of columnar and goblet cell) of a lepidopteran (moth) larva. Although the final microvilli preparation was enriched with some putative enzyme markers, contamination by cell membranes of the goblet cell could not be ruled out by the lack of microscopic data. Goblet cells are characterized by having modified microvilli with mitochondria inside them.

This paper reviews data on plasma membranes from midgut cells taking into account cell types, midgut regions and the phylogenetic position of the insect. Throughout, the focus is on providing a coherent picture of data and highlighting further research areas.

MICROVILLAR AND BASOLATERAL MEMBRANES

Isolation of Membranes from the Midguts of Different Insects

The insect migut cell microvillus is homologous to that described in vertebrates and reviewed by Bement and Mooseker (1996). Thus, a bundle of parallel actin filaments cross-linked by the actin-bundling proteins villin and fimbrin form the core of a microvillus. Lateral side arms (composed of myosin I and the Ca^{2+}-binding protein, calmodulin) connect the sides of the actin bundle to the overlying plasma membrane. The actin bundles from the microvillus extend down into the cell and are rooted in the terminal web, where they are linked together by a set of proteins including spectrin and myosin II (Bautz 1989, Bonfanti et al. 1992, Morgan et al. 1995, Dallai et al. 1998).

After the development of a method to isolate microvilli from midgut caeca cells from a lower Diptera (see above), Cioffi and Wolfersberger (1983) devised an ingenious (although tedious) method to isolate plasma membranes from the midgut columnar and goblet cells of lepidopteran larvae, based on the stepwise disruption of the midgut by ultrasound. This method was used to recognize enzyme markers (most of them digestive enzymes) for columnar cell microvilli (Wolfersberger 1984) and to show that H^{+}/K^{+}-ATPase is located exclusively in the modified microvilli of the midgut goblet cells (Wieczorek et al. 1984). Cioffi and Wolfersberger (1983) were also able to isolate lateral and basal membranes from lepidopteran midgut cells.

Santos et al. (1986) compared several procedures to prepare microvilli from lepidopteran midgut columnar cells with electron microscope monitoring. They showed that, although preparations obtained by the ultrasound technique are almost free from contaminants, the yield of microvillar membranes is very low compared with the divalent cation differential precipitation methods. After this paper and complementary data from Eisen et al. (1989), differential precipitation methods became the method of choice to prepare microvillar membranes from columnar cells of lepidopteran midguts. Those preparations are used to study membrane-bound digestive enzymes, transport phenomena and the binding of toxins (see below).

In addition to lower Diptera and Lepidoptera, cation differential precipitation has been used to isolate microvilli from midgut cells from other insect orders such as Dictyoptera (cockroaches) (Parenti et al. 1986), Coleoptera (beetles) (Ferreira et al. 1990), and higher Diptera (flies) (Lemos and Terra 1992).

Electron microscopy of insect midgut microvilli preparations (Houk et al. 1986, Santos et al. 1986) demonstrates, as previously observed for vertebrate enterocytes (Schmitz et al. 1973), that they are substantially free from other cell structures, although the microvilli still contain some cytoskeleton elements. The amount of contaminating membranes is evaluated with marker enzymes. It should be noted, however, that many enzyme markers of subcellular structures, with the exception of succinate dehydrogenase (mitochondria) and lactate dehydrogenase (cytosol), are not always suitable. Thus, γ-glutamyl transferase, which is a useful microvillar membrane marker for Diptera (Bodnaryk et al. 1974, Espinoza-Fuentes et al. 1987), Lepidoptera (Eisen et al. 1989) and Dictyoptera (Parenti et al. 1986), occurs only in trace amounts in Coleoptera (Ferreira et al. 1990) and only in soluble form in Hymenoptera (bees, wasps, and ants) (Schumaker et al. 1993). Alkaline phosphatase, a plasma membrane (microvillar or microvillar plus basolateral membranes) marker in most insects (Terra and Ferreira 1994), is a soluble enzyme in larvae of Coleoptera (Ferreira et al. 1990, Reuveni et al. 1993) and in adults of Diptera (Houk et al. 1986) and Lepidoptera (Terra et al. 1990). Acid phosphatase, which is a marker of lysosomes in some tissue (e.g. mammalian liver, Evans 1978), is found mainly in the cytosol of larval midgut cells of Diptera (Ferreira and Terra 1980), Lepidoptera (Santos and Terra 1984) and Coleoptera (Ferreira et al. 1990).

The enrichment of microvillar membranes in a preparation depends on the ratio of total cellular protein to microvillar protein. The lower the microvillar protein concentration relative to total protein, the more enrichment of microvillar membranes can occur. Thus, higher enrichments may result from small microvilli relative to cell size, of microvillar membranes poor in protein components or of the fact that only parts of the cells of the tissue have true microvilli. As a consequence, enrichments vary widely according to midgut region and to the phylogenetic group the insect pertains (see review in Terra and Ferreira 1994).

Biochemistry of Microvillar Membranes

Early attempts to study the biochemistry of microvillar membranes consisted on the determination of the ratio of phosphorus to protein content in microvilli preparations from Lepidoptera larvae and SDS-PAGE of these proteins (Wolfersberger et al. 1987) and in similar preparations from Diptera adults (Houk et al. 1986). Nevertheless, as discussed above, microvillar membranes are contaminated by cytoskeleton elements and other minor components. Jordão et al. (1995) prepared microvilli from midguts of *Rhynchosciara americana* and *Musca domestica* (a lower and higher Diptera, respectively) and *Tenebrio molitor* (Coleoptera) using the calcium differential precipitation method. The microvilli were then treated with hyperosmotic Tris buffer that disrupts microvilli into microvillar membranes and core material. On dilution, the core material dissociates, permitting the pelleting of microvillar membranes, while leaving cytoskeleton elements in the supernatant. The microvillar membranes were shown to be free from contaminating membranes and cytoskeleton elements by chromatography in Sepharose 4B, SDS-PAGE and electron microscopy. A similar approach was used to prepare microvillar membranes free from cytoskeleton elements from *Spodoptera frugiperda* (Lepidoptera) (Capella et al. 1997). Specific activities of marker enzymes are 1.5- to 2.5–fold higher than in the original microvilli preparations, which is not different from the best preparations from mammals.

The density of the purified insect midgut microvillar membranes linearly increase with the protein-lipid mass ratio (Fig. 1). Nevertheless, *T. molitor* datum is remarkably low and should be reinvestigated. The observed range of protein-lipid mass of insect microvillar membranes is 1.41–3.13, which is wider than that found among mammalian enterocytes (1.54–2.44, Proulx 1991).

Apparently there is an inverse relationship between protein-lipid mass ratio (or membrane density) and cholesterol and carbohydrate content in insect microvillar membranes. Thus, protein-

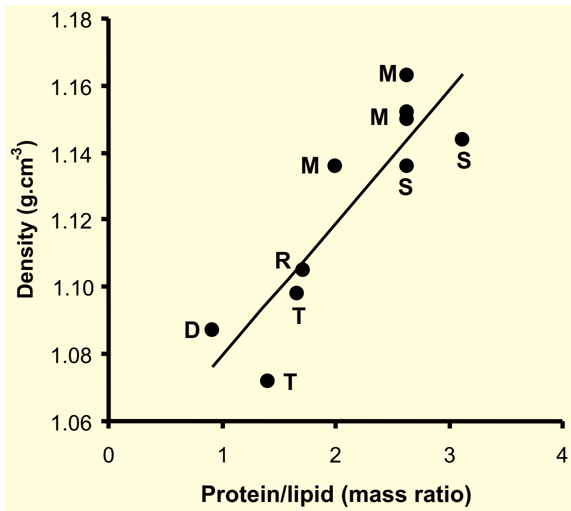

Fig. 1 – Densities and protein-lipid mass ratios of purified midgut microvillar membranes from different insects. Densities were taken from Table I and the corresponding protein-lipid ratios from the cited literature. The three heaviest membranes were from *M. domestica* posterior midgut and their densities were averaged before displaying in Table I. D, *Dysdercus peruvianus*; M, *Musca domestica*, R, *Rhynchosciara americana*; S, *Spodoptera frugiperda*; T, *Tenebrio molitor*.

lipid mass ratio, carbohydrate (μg/mg protein) and cholesterol (μg/mg protein) contents are, respectively: 1.4–1.7, 400–700, and 110–140 for Coleoptera; 2.0–2.6, 240–410, and 40–59 for higher Diptera; 2.6–3.8, 0–80, and 17–28 for Lepidoptera (Jordão et al. 1995, Capella et al. 1997). Lipids in insect microvillar membranes are supposed to be (total phosphorus data) phospholipids (Jordão et al. 1995), in accordance with similar data for mammalian enterocytes (Proulx 1991). It is not possible, however, to discount that in insects other than lepidopterans glycolipids may be important components of membranes. In this case, part of the phosphorus found in membranes would occur in proteins and carbohydrates (Jordão et al. 1995, Capella et al. 1997).

A detailed study of the chemical composition of microvilli (microvillar membranes plus contaminating cytoskeleton) from *Bombyx mori* midgut cells (Leonardi et al. 2001) confirms the previous data. Thus, protein-lipid ratio is smaller (1.85) in anterior plus middle in comparison to posterior midgut (2.30). Phospholipids account for 77% (phosphatides add to 62%) of total lipids with glycolipids summing 8%.

As densities are a valuable parameter in membrane characterization, such determinations were carried out in representative insects and the results were compiled in Table I. Densities were determined by sucrose-density-gradient centrifugation with aminopeptidase as enzyme marker, which revealed a contamination of microvillar membranes by a lighter membrane amounting to 5% of the total membranes. These lighter membranes were supposed to be basolateral membranes having an integral aminopeptidase, as basolateral membranes of mammalian enterocytes (Maroux et al. 1988). Curiously enough, trehalase assays showed that in microvillar membrane preparations from *S. frugiperda* midgut cells there are membranes lighter (anterior midgut: 1.061; posterior midgut: 1.057) than those found with aminopeptidase assays shown in Table I.

This suggests that the sucrose gradients are resolving more than one domain of basolateral membranes. Microvillar densities vary widely among insects, with more evolved ones (Lepidoptera and Diptera) having membranes with densities higher than 1.135. This suggests that the midgut cell surface plays more sophisticated roles (associated with a higher protein content) than in lower insects. The same is true for microvillar in comparison to basolateral membranes. What kind of roles these membranes play will be discussed in the next section.

Physiological Role of Microvillar and Basolateral Membranes

Initial considerations and surface digestion

The physiological role of midgut microvillar membranes may change along the midgut and among insect taxa and should include: surface (terminal) digestion, absorption, ion homeostasis, signaling, and unique digestive enzyme secretion mechanisms. Some of these functions depend on the concurrent participation of basolateral membranes, like those associated with the transepithelial transport of water, ions and nutrients. Most of the membrane roles are played by integral membrane (occasionally cytoskeleton) proteins that will be considered in turn.

The densities of isolated plasma membranes of insect cells depend essentially on their protein contents (Fig. 1). If we set appart data on *P. americana* (not purified microvillar membranes) and on hemipterans (to be discussed on section 4), the amount of protein in membranes may largely reflect digestive enzyme content, in accordance with the presumed role of these membranes in digestion. Thus, in Coleoptera most digestion occurs inside the peritrophic membrane (cylindrical anatomical structure separating the midgut contents from the midgut cells) with little or no digestion being carried out by enzymes associated with the microvillar membranes. In contrast, in Diptera the initial and intermediate stages of digestion occur in midgut lumen and most terminal digestion is carried out by microvillar enzymes. Furthermore, there is a differentiation along the Coleoptera and higher Diptera midguts so that most terminal digestion takes place at the posterior midgut, which in higher Diptera functionally corresponds to the whole midgut of other insect species (Terra and Ferreira 1994, 2005).

Pyrearinus termitilluminans larvae regurgitate onto their prey their midgut contents that accomplishes initial digestion. Pre-liquefied material is then ingested by larvae and the intermediate and final digestion take place on the surface of midgut cells by microvillar enzymes (Colepicolo-Neto et al. 1986), thus explaining the high density of microvillar membranes. It is not clear why the microvillar membranes in *Z. subfasciatus* midgut cells are heavy, because most digestion in these insects occurs in luminal contents (Silva et al. 1999). One possibility is contamination of the microvillar membranes by peritrophic gel, a not well-known substance that replaces the peritrophic membrane in these insects (Terra 2001).

Lepidopteran data (Table I) clearly do not follow the rule according to which the amount of protein in membranes reflects digestive enzyme content. In these insects, enzymes involved in terminal digestion are immobilized at the surface of midgut cells because they are entrapped in the cell glycocalyx, instead of being integral membrane proteins (Terra and Ferreira 1994, 2005). The high density of lepidopteran midgut microvillar membranes probably results from a large amount of different transporters, although it is not clear why lepidopteran larvae need more transporters than dipteran larvae.

Microvillar integral digestive enzymes vary among different taxa. Most frequently they are: aminopeptidase, alkaline phosphatase, carboxypeptidase, dipeptidase, and α-glucosidase (Terra and Ferreira 1994). For a recent review of these enzymes see specific entries in Terra and Ferreira (2005).

Ion and water transport

Absorption of nutrients and ions is carried out by membrane integral proteins known as transporters.

TABLE I

Densities ($g.cm^{-3}$) of isolated microvillar (MVM), perimicrovillar (PMVM) and basolateral (BLM) membranes of insect midgut cells[1].

Insect (Order)	Midgut region	MVM	PMVM	BLM	Reference
P. americana (Dyc)	Anterior	1.092[2]	–	1.056	This paper
	Posterior	1.154[2]	–	1.081	This paper
D. peruvianus (Hem)	Whole	1.132	1.087	1.064	Silva et al. 1996
R. prolixus (Hem)	Whole	1.086	1.068	n.d.	Ferreira et al. 1988
A. pisum (Hem)	Whole	1.153	1.138	1.117	Cristofoletti et al. 2003
T. molitor (Col)	Anterior	1.072	–	n.d.	Jordão et al. 1995
	Posterior	1.098	–	n.d.	Jordão et al. 1995
P. termitillum. (Col)	Whole	1.168	–	1.128	This paper
Z. subfasciatus (Col)	Whole	1.170	–	1.120	This paper
M. frianus (Col)	Whole	1.116	–	1.107	This paper
M. domestica (Dip)	Anterior	1.136	–	1.113	Jordão et al. 1995
	Posterior	1.155[3]	–	1.110[3]	Jordão et al. 1995
R. americana (Dip)	Caeca	1.105	–	n.d.	This paper
D. saccharalis (Lep)	Anterior	1.155	–	1.075	This paper
	Posterior	1.162	–	1.074	This paper
S. frugiperda (Lep)	Anterior	1.136	–	1.068	Capella et al. 1997
	Posterior	1.144	–	1.065	Capella et al. 1997

[1]Procedures used in the determinations were described in the corresponding references, whereas those employed in this paper were as follows. Purified microvillar membranes (no cytoskeleton) were prepared by Tris disruption from microvillus samples obtained by Mg^{++}-differential precipitation of midgut tissue homogenates, according to Capella et al. (1997). Microvillar membranes were resolved in 10 ml sucrose gradients (10–60%) prepared in 50 mM Tris-HCl, pH 7.0. Aminopeptidase was assayed as a marker enzyme and densities were calculated from the refractive index of gradient fractions as detailed in Capella et al. (1997). The densities of the basolateral membranes for *M. domestica* and *S. frugiperda* were calculated from the authors' data. Col, Coleoptera; Dip, Diptera; Dyc, Dyctioptera; Hem, Hemiptera; Lep, Lepidoptera; *P. termitillum*, *P. termitilluminans*; -, structure absent; n.d., not determinant. – [2]Microvilli samples, not purified microvillar membranes. – [3]Average of densities of membranes from 3 sections of the posterior midgut.

Ion transporters may be ion-motive ATPases, antiporters, and symporters. Ion-motive ATPases are divided into three distinct families: F-, V-, and P-type ATPases (Pedersen and Carafoli 1987a). The F-type ATPases are proton ATPases found in the inner mitochondrial membrane and are not relevant here.

The V-type (vacuolar-type) ATPases (H^+ V-ATPases) are critical for receptor recycling pathways by acidifying endocytotic vesicles and promoting receptor-ligand dissociation. They are also found in epithelia of vertebrate kidney, lepidopteran midgut globlet cells and insect Malpighian tubules (Forgac 1999, Nishi and Forgac 2002). The H^+ V-ATPase has two distinct sectors: the membrane-bound V_o sector and the intracellular V_1 sector. The last one corresponds to stalked structures in the cytoplasmic face of insect epithelia called "portasomes" (Forgac 1999, Nishi and Forgac 2002, Wieczorek et al. 2003, Rizzo et al. 2003). The H^+ V-ATPase, which is bafilomycin-sensitive, transports H^+ and activates secondary transport processes, exemplified by K^+-amino acid symport (see below), fluid secretion by Malpighian tubules (see water transport below), or midgut luminal alkalinization (see Modified Microvillar Membranes).

P-type (or E_1E_2 type) ATPases are so called because the enzyme works via a covalent, phosphorylated intermediate. Because of this, P-type ATPases can generally be inhibited by vanadate, which binds to the phosphate-binding site and thus blocks the phosphorylation cycle of the enzyme (Pedersen and Carafoli 1987a). They use the energy released from ATP hydrolysis to drive the membrane transport of mono- and divalent ions (Horisberger et al. 1991). Eukaryotic P-type ATPases include ouabain-sensitive Na^+/K^+-ATPases of the plasma membrane of multicellular animals (including insects, see Emery et al. 1998), the H^+/K^+-ATPases of gastric and colon of mammals (Pedersen and Carafoli 1987b) and higher Diptera middle midguts (see 3). The Na^+/K^+-ATPase is responsible for maintenance of electrochemical gradients across the plasma membrane by exporting 3 Na^+ from the cell and importing $2K^+$ to the cell during each reaction cycle. The H^+/K^+-ATPase pumps H^+ and is most closely related structurally to Na^+/K^+-ATPase (Maeda et al. 1990).

Other ion-transporters found in insects are: amiloride-sensitive Na^+ or K^+/H^+-antiporter, amiloride-resistant Na^+/H^+-antiporter, and bumetanide-sensitive $Na^+/K^+/Cl^-$symporter (Pullikuth et al. 2003). The putative mechanism (Pullikuth et al. 2003) for fluid secretion by mosquito Malpighian tubules illustrates how the ion-transporters may have a concerted role. According to this mechanism, an apical H^+ V-ATPase pumps H^+ ions that are exchanged by Na^+ or K^+ ions through an apical amiloride-sensitive Na^+ or K^+/H^+-antiporter, resulting in fluid secretion with the presumed help of aquaporins (Borgnia et al. 1999). Basolateral transport activated through cAMP (formed by the action of diuretic peptides onto basolateral membrane receptors) involve a $Na^+/K^+/Cl^-$ symporter and an amiloride-resistant Na^+/H^+-antiporter. Some Malpighian tubular cells have also a basal Na^+/K^+-ATPase.

Most insects have a countercurrent flux of fluid in their midguts caused by secretion of fluid in the posterior midgut and its absorption in anterior midgut (Terra and Ferreira 1994, 2005). The mechanism described above for mosquitoes may serve as a model for fluid secretion in posterior midgut of most insects. The absorption of fluid in the insect anterior midgut lacks a model incorporating molecular features and discussion on this subject relies on morphological observations. The cells in insect anterior midgut usually display basal plasma membrane infoldings modified into long and narrow channels with particles attached to their cytoplasmic side (e.g. interstitial cell in higher Diptera, Terra et al. 1988) or modified into short and ramified channels with few apertures to the extracellular space (e.g. lepidopterans, Santos et al. 1984). The fact that these infoldings constitute an extracellular compartment, which has restricted access to the hemolynph (due to the restricted openings into the underlying extracellular space), should permit the cell to concentrate solutes in that compartment to create an osmotic pressure gradient between compartment and lumen, which might assist the absorption of water.

In addition to their involvement in the transepithelial transport of solutes and water, basolateral membranes may have other functions. The presence of several digestive enzymes in these membranes is not understood, but trehalase may play a role in the midgut utilization of hemolymph trehalose (Azuma and Yamashita 1985.

Sugar absorption

In mammalian enterocytes, there are two kinds of glucose transporters: the Na^+/glucose cotransporter, or symporter (SGLT1), and the facilitative transporter, or uniporter (GLUT2). The symporter is strictly dependent on the presence of Na^+, inhibited by phloridzin and is found in the enterocyte apical membrane. In contrast, the uniporter is inhibited by phloretin and cytochalisin B and is localized at the enterocyte basal membrane (Hediger et al. 1987, Takata 1996, Kellett 2001, Giordana et al. 2003).

Although it has not been functionally demonstrated the presence of sugar transporters in insect midgut cells, a glucose uniporter is very likely to occur. *Drosophila melanogaster* has a glucose transporter gene that is homologous with the mammalian glucose uniporter genes (Escher and Rasmuson-Lestander 1999). Furthermore, the absorption of glucose by the epidermis of the endoparasitoid *Aphidius ervi* seem to involve a uniporter (Giordana et al. 2003).

Amino acid transport

Amino acid transporters have been studied in the midguts of adult stage of the dictyopteran (cockroach) *Blabera gigantea* and in the larval stage of the coleopteran *Leptinotarsa decemlineata* and the lepidopterans *Philosamia cynthia*, and *Bombyx mori*. In coleopterans, only uniporters were found, whereas in the other insects amino acid-cation symporters occur in addition to the uniporter. Dictyopterans have a Na^+-coupled amino acid transport with the electrochemical potential maintained by a Na^+/K^+-ATPase such in mammalian cells. Lepidopterans possess transport features different from those of mammals. K^+ is secreted in these insects through the concerted action of an apical H^+V-ATPase and apical K^+/H^+-antiporter, thereby providing the drive force for absorption of amino acids by an amino acid-K^+ symporter (Castagna et al. 1997).

The successful preparation of vesicles from lepidopteran midgut microvillar membranes (brush-border membrane vesicles) (see Isolation of Membranes from Midgut of Different Insects) prompted the study of amino acid transport, beginning with the paper of Hanozet et al. (1980). The studies led to the finding of several distinct amino acid-K^+ symporters with overlapping specifies (Castagna et al. 1997, Wolfersberger 2000). A cDNA encoding a lepidopteran midgut amino acid-K^+ symporter was cloned. The encoded protein showed weak but significant sequence identity with amino acid transporters belonging to the sodium-dependent and chloride-dependent γ-aminobutyric acid (GABA) superfamily (Castagna et al. 1998). Since then, the sequence of several new insect cation-amino acid symporters were deposited in the GenBank and most of the present research looks for specific inhibitors of amino acid transport like bestatin, (previously widely used as an aminopeptidase inhibitor) and fenoxycarb (Wolfersberger 2000).

Secretory mechanisms

The microvillar molecular organization is probably modified in insects displaying unique secretory mechanisms not seen in other animals. Lepidopteran anterior midgut cells secrete digestive enzymes by two kinds of microapocrine secretion. In the microapocrine secretion with budding vesicles, small vesicles migrate into the microvilli, from which they bud laterally as double membrane vesicles. Microapocrine secretion with pinched-off vesicles is characterized by vesicles migrating to the tips of microvilli, where they fuse one another and with the microvillar membrane. Finally, vesicles pinch off from the enlarged microvilli tips. In both cases, the secretory contents are released by membrane fusion and/or by membrane solubilization caused by high pH contents or by luminal detergents (Terra and Ferreira 1994, 2005).

In accordance with the putative microvillar cytoskeleton differences associated with unique secretory mechanisms, lepidopteran anterior midgut microvilli preparations are free from cytoskeleton before the Tris-disruption step (see Isolation of Membranes from Midgut of Different Insects and Biochemistry of Microvillar Membranes) (Capella et al. 1997). A *S. frugiperda* midgut cDNA expression library is being screened with antibodies raised with purified microvillar membranes as antigens, in an attempt to identify the molecules involved in these secretory mechanisms (A.H.P. Ferreira, L.O. Guerra, P.B. Paiva, B. Schnabel, M.R.S. Briones, W.R. Terra and C. Ferreira, unpublished data).

MODIFIED MICROVILLAR MEMBRANES

Modified microvilli are typical of the lepidopteran goblet cells and higher dipteran oxyntic cells. Goblet cells have a cavity, which is formed by invagination of the apical membrane and which occupies most of the cell (long-neck goblet cell) or only its upper part (stalked goblet cell). The infolded apical membrane shows modified microvilli containing mitochondria and their cytoplasmic side are studded with small particles (Cioffi 1979, Santos et al. 1984) that corresponds to a H^{+}V-ATPase (Wieczorek et al. 2003). Goblet cells generate a high gut pH in lepidopterans according to the following model (Wieczorek et al. 2003). Carbonic anhydrase produces carbonic acid that dissociates into bicarbonate and a proton. The proton is pumped by an H^{+}V-ATPase into the goblet cell cavity, from where it is removed in exchange with K^{+} that eventually diffuses into lumen. Bicarbonate is secreted in exchange with chloride and loses a proton due to the intense field near the membrane, forming carbonate and raising the gut pH.

Oxyntic cells have an apical membrane invaginated into ramified crypts which are coated with microvilli and display numerous mitochondria in their cytoplasm. The cytoplasmic side of oxyntic microvillar membranes are studded with small particles (Terra et al. 1988). These are believed to be proton pumps (P-type ATPase, see *Ion and water transport*) that acidify to pH 3.2 the contents of middle midgut in higher Diptera. Chloride ions seem to follow the movement of protons. This hypothesis is supported by the observed effect of different compounds in luminal pH and in the luminal chloride content (Terra and Regel 1995). It is remarkable how similar is the mechanism of insect midgut luminal acidification and that one found in mammalian stomachs (Forte et al. 1980).

PERIMICROVILLAR MEMBRANES

Perimicrovillar membranes (PMVM) are membranes that cover the midgut cells microvilli extending into the gut lumen with dead ends (Lane and Harrison 1979, Andries and Torpier 1982, Silva et al. 1995) and were described in Hemiptera (bugs, aphids, and cicadas). The domain of PMVM ensheathing the microvilli are set in position by columns obliquely disposed between them and the microvillar membrane (Lane and Harrison 1979).

Freeze-fracture replicas showed that PMVM are almost free from intramembranous particles, thus resembling myelin sheets (Lane and Harrison 1979, Andries and Torpier 1982). Therefore, PMVM must display a lower buoyant density than the microvillar membranes. Based on this, the two membranes were isolated by density-gradient centrifugation and enzyme markers identified: α-glucosidase for PMVM and α-mannosidase or β-glucosidase for microvillar membranes (Ferreira et al. 1988, Silva et al. 1996). PMVM densities of *R. prolixus* and *D. peruvianus* are alike that of myelin sheets, as expected, although *A. pisum* PMVM are surprisingly heavy (Table I). It should be noted, however, that the last mentioned PMVM are actually modified PMVM and seem to have a high enzyme content (see below). Microvillar membrane densities of *R. prolixus* are small in contrast to that of *D. peruvianus* and *A. pisum* (Table I). This probably reflects the putative pumps seen in *D. peruvianus* microvillar membranes and the contamination of microvillar membranes by lamellar links in *A. pisum* (see below). Immunolocalization of the PMVM enzyme marker, α-glucosidase, suggests that these membranes are formed when double membrane vesicles fuse their outer membranes with the microvillar membranes and their inner membranes with PMVM. A double membrane Golgi cisterna (on budding) forms the double membrane vesicles (Silva et al. 1995).

PMVM and a PMVM-bound α-glucosidase occur in the major hemipteran infra-orders and in the sister order Thysanoptera (thrips), but lack in the orders Psocoptera (plant lice) and Phthiraptera (lice). This suggests that PMVM may have originated in the condylognatha (Paraneopteran taxon including Hemiptera and Thysanoptera) ancestral stock (Silva et al. 2004). The Condylognathan ancestors should feed as present-day thrips on phloem by a punch and suck mechanism. Phloem has very low contents of protein (with few exceptions) and carbohydrate polymers and is rich in sucrose and relatively poor in free amino acids (Terra 1990). Upon adapting to this food, Condylognathan ancestors would lose most digestive enzymes and the peritrophic membrane that are associated with luminal digestion. Essential amino acids present in low concentrations in sap may be absorbed by a hypothesized mechanism (Terra 1988, Terra and Ferreira 1994) as follows: microvillar membranes actively transport K^+ from the perimicrovillar space into the midgut cells, generating a concentration gradient between the gut luminal sap rich in K^+ and the perimicrovillar space. This concentration gradient may be the driving force for the active absorption of amino acids by appropriate symporters in PMVM. Amino acids, once in the perimicrovillar space may diffuse up to specific transporters on the microvillar surface. Although amino acid symporters have been found in the microvillar membranes of several insects (see *Amino acid transport*), no attempts have been made to study the other postulated proteins (e.g., amino acid-K^+-symporters in PMVM and potassium pumps in microvillar membranes). Thus, in spite of the model provided an explanation for the occurrence of these peculiar cell structures in condylognatha, it is supported only by: (1) evidence that amino acids are absorbed with potassium ions in *Dysdercus peruvianus* (Silva and Terra 1994); (2) occurrence of particles studying the cytoplasmic face of the midgut microvillar membrane of *D. peruvianus*. These might be ion pumps responsible for the putative K^+-transport (Silva et al. 1995).

Organic compounds in xylem sap (much more diluted than phloem sap) need to be concentrated before they can be absorbed by the perimicrovillar membrane. This occurs in the filter chamber that consists of a thin-walled, dilated anterior midgut in close contact with the posterior midgut and the proximal ends of the Malpighian tubules (anatomical structure analogous to the mammalian nephron). This arrangement enables water to pass directly from the anterior midgut to the Malpighian tubules, concentrating food in midgut. The high permeability of the filter chamber membrane to water results from the occurrence of specific proteins named aquaporins (Borgnia et al. 1999). These were immunolocalized in the microvillar border (PMVM and microvillar membranes) of the filter chamber cells of several hemipteran xylem sap feeders (Le Cahérec et al. 1997).

Hemipterans like aphids may suck high-sucrose phloem saps with osmolarity up to three times that of the insect hemolymph. This results in a considerable hydrostatic pressure caused by the tendency of water to move from the hemolymph into midgut lumen. To withstand these high hydrostatic pressures there are links between apical lamellae (replacing usual midgut cell microvilli). As a consequence of these links, PMVM could no longer exist and were replaced by membranes seen associated with the tips of the lamellae, the modified PMVM, which contain unexpected enzymes like a cysteine proteinase (Ponsen 1991, Cristofoletti et al. 2003).

Haematophagous, seed-sucking, and predator hemipterans evolved from the sap-feeders regaining the ability to digest polymers. Compartmentalization of digestion was maintained by PMVM as a substitute for the absent peritrophic membrane (Ferreira et al. 1988, Silva et al. 1995).

CONCLUDING REMARKS

The study of the plasma membranes of insect midgut cells has progressed enough to reveal many of their characteristics. Research emphasis on the unique aspects of insect midgut cells may led to seminal findings, in disparate fields as cell biology, molecular physiology, and molecular evolution as may provide new targets for insect control. Thus, the study of microvilli engaged in microaprocrine secretion may disclose novel mechanisms of vesicle trafficking and membrane fusion. The description in molecular detail of the plasma membrane role in the functioning of oxyntic and interstitial cells from the midgut of higher Diptera should illustrate a marvelous case of convergence with mammalian gastric cells. A molecular physiological approach to the interplay of hemipteran midgut plasma membranes will certainly be revealing, as these insects are the only animals that live exclusively sucking the usually nutrient-poor plant saps. Finally, the implications of the knowledge on the plasma membrane signaling system of midgut-function coordination is beyond speculation, due to scarcity of data. Progress in the fields reviewed is being supported by the association of biochemical and molecular biology procedures. A proteomic approach to those fields is still hampered by the lack of sufficient biological material from specific insect tissues.

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RESUMO

As membranas plasmáticas das células intestinais dos insetos apresentam um domínio apical e outro basal. O domínio apical é geralmente modificado em microvilosidades com organização molecular similar a de outros animais, embora possam diferir naqueles insetos que apresentam vesículas secretoras em trânsito que brotam lateralmente ou destacam-se das extremidades das microvilosidades. Outras modificações microvilares estão associadas a bombeamento de prótons ou a interrelações com uma membrana lipídica (a membrana perimicrovilar) que reveste as microvilosidades de células intestinais de hemípteros (pulgões e percevejos). Admite-se que as membranas perimicrovilares estejam envolvidas na absorção de aminoácidos a partir de dietas diluídas. As membranas microvilares e perimicrovilares tem densidades distintas (e conteúdo protéico) que dependem do táxon do inseto. O papel desempenhado pelas proteínas microvilares e perimicrovilares na fisiologia intestinal dos insetos é revisto, procurando fornecer uma visão coerente dos dados e chamando a atenção para novos objetivos de pesquisa.

Palavras-chave: membranas microvilares, membranas perimicrovilares, absorção de nutrientes, transporte de íon, transporte de água, fisiologia molecular do intestino médio.

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