Dynamics of Ultrastructural Characters of *Drosophyllum lusitanicum* Link (Droseraceae) Digestive Glands During Maturation and After Stimulation

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ABSTRACT: A quantitative investigation was conducted on the endomembrane system of the secretory cells of *Drosophyllum lusitanicum* Link digestive glands exporting acid hydrolases. The surface density of rough endoplasmic reticulum (RER) membranes as well as the number of Golgi stacks, clathrin-coated vesicles (CCVs) and their smooth derivatives increased progressively with the gland maturation. On average secretory cells of the fully mature unstimulated gland, each contained RER with a surface area of 17500 μm^2 and 16000 small vesicles (including CCVs of 81 nm average diameter and smooth vesicles as the derivatives of CCVs which average 46 nm). One hour after stimulation imitating prey capture, the surface density of RER, the number of Golgi stacks and "protein" vesicles all increased greatly. The data obtained indicate that smooth vesicular derivatives of CCVs are involved in the secretion of digestive enzymes. Hydrolase secretion started in the immature glands and continued in the mature stage irrespective of whether or not an insect was actually captured. Stimulation resulting in the release of digestive fluid on the surface of the leaf by the digestive glands appeared to be accompanied by a significant increase in the rate of hydrolase synthesis and secretion. In addition to protein vesicles, two other types of larger secretory Golgi vesicles were produced: granular-fibrillar ones (~ 105 nm in diameter that were confined to cells at the meristem stage), and loosely-fibrillar vesicles (~ 250 nm in diameter that occurred only at the stage of wall ingrowth deposition). Models are proposed that depict digestive gland cell functioning both before and after stimulation.

KEY WORDS: **Acid hydrolase secretion, carnivorous plants, coated vesicles, *Drosophyllum*, morphometry, ultrastructure.**

INTRODUCTION

Digestive glands of carnivorous plants including *Drosophyllum lusitanicum* synthesize and secrete various hydrolases operating optimally at an acidic pH (Heslop-Harrison, 1975; Vassilyev, 1977; Juniper *et al.*, 1989; Higashi *et al.*, 1993). In unstimulated glands the enzymes are usually stored in the cell walls and, to a lesser extent, in some (lytic) vacuoles.

The study of several types of animal and plant cells (but not plant glands) showed that acid hydrolases are synthesized on membrane-bound polysomes of the rough endoplasmic reticulum (RER), and processed through Golgi stacks and *trans*-Golgi networks (TGNs, a vesicle sorting apparatus). Thereafter, enzymes are packaged into clathrin-coated vesicles (CCVs) that are structures of both plant and animal cells specialized for selective loading and intracellular trafficking of acid hydrolases (reviewed by Schmid, 1997; Neuhaus and Rogers,

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Abbreviations: CCV = clathrin-coated vesicle, PV = protein vesicle (common name for CCV and its smooth derivative), RER = rough endoplasmic reticulum, TGN = *trans*-Golgi network.

1998; Robinson *et al.*, 1998; Nebenführ and Staehelin, 2001; Vitale and Galili, 2001). CCVs produced by the TGNs are very small structures, usually 60-80 nm in diameter (Coleman *et al.*, 1987; Beevers, 1996). Clathrin is a cytosolic protein that forms a characteristic lattice on the surface of the vesicle membrane. In sectional views the lattice appears as a series of short spikes radiating from the membrane (Scales *et al.*, 2000). The role of the coat is assumed to be involved in the generation of a mechanical force required to form an evagination of the TGN membrane and then to pinch it off as a free vesicle. Soon after vesicular detachment the coat dissociates and the vesicles become smooth-surfaced with an outer diameter of about 50 nm. There is evidence that in both animal (reviewed by Le Borgne and Hoflack, 1998) and plant (reviewed by Hillmer *et al.*, 2001; Vitale and Galili, 2001) cells the CCVs mediate transfer of acid hydrolases to the lysosomes including lytic vacuoles.

Based on the above information, it may be asked if CCVs and their smooth derivatives in the digestive gland cells of carnivorous plants are involved in acid hydrolase loading not only to vacuoles but also to the cell walls, the main site of their accumulation in secretory cells? In order to answer this question, an ultrastructural morphometric study of the digestive glands of *D. lusitanicum* (Droseraceae) in various developmental and artificially stimulated states was made. Special attention was given to the dynamics of the endomembrane system, including RER, Golgi apparatus, CCVs, their smooth derivatives and other secretory Golgi vesicles. The results obtained give a positive answer to the question asked above.

It should be remembered here the pioneering work by Schnepf (1960, 1961) who was the first to apply morphometry to the study of secretory cell ultrastructure. He counted dictyosomes, plastids, mitochondria and Golgi vesicles per 100 μm^2 of sectioned secretory cells in digestive and slime glands of *D. lusitanicum* unstimulated leaves. Schnepf was able to find secretory vesicles only in the alluring slime-producing cells.

MATERIALS AND METHODS

Plants were greenhouse grown. The digestive glands on the leaves of *Drosophyllum lusitanicum* were studied in six states, (1) meristematic (before completion of cell division), (2) immature (at the onset of cell wall ingrowth deposition), (3) mature unstimulated, (4), (5) and (6) mature and stimulated for 1, 4 and 8 h respectively. The stimulation aimed to imitate prey capture was made by placing droplets of egg albumin on leaves (see Vassilyev, 2002). The secretion of digestive fluids appeared on the leaf surface soon after stimulation and was in full activity after 30 min. The leaf portions were fixed in glutaraldehyde followed by osmium tetroxide and embedded in an araldite-epon mixture according to Vassilyev (1977). Ultrathin sections of the digestive glands were examined and photographed using a Hitachi H-600 transmission electron microscope.

Morphometric studies were performed according to the procedure of Steer (1981), using printed micrographs of the outer secretory cells of the digestive glands. Detailed description of the procedure is given elsewhere (Vassilyev and Muravnik, 1997; Vassilyev, 2000a). The mean volume of cells was calculated by multiplying the surface area of their sections to the length of their periclinal wall and checked using volume density and absolute volume of the nucleus. For the demarcation of the cisternae in a dictyosome the criteria were based on those given by Staehelin *et al.* (1990). Standard errors were calculated for all values directly determined on electron micrographs.

RESULTS AND DISCUSSION

There are two types of leaf glands in *Drosophyllum lusitanicum*, stalked alluring slime-producing glands and sessile digestive glands. The digestive glands consist of four cell tiers, outer and inner secretory cells, barrier cells and basal cells (Fig. 1). The secretory cells are differentiated as transfer cells. They bear numerous wall ingrowths that are confined to the inner periclinal wall and inner portion of the anticlinal walls in the outer layer of cells (Fig. 2). In the inner layer, they extend along the entire inner cell surface. There are specific pores in the cuticle of the outer cells (Vassilyev, 2002). The barrier (endodermoid) cells develop fully cutinized Casparian strips on their anticlinal walls. The cells of the glands are interconnected with each other and with neighboring cells by plasmodesmata (Vassilyev, 2002).
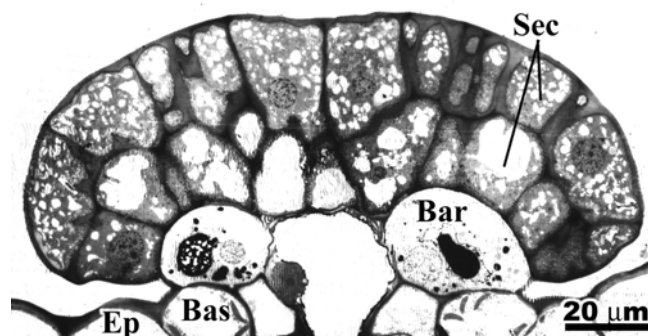

Fig. 1. General sectional view of the digestive gland in *Drosophyllum lusitanicum*. Bar: barrier cell; Bas: basal cell; Ep: epidermis; Sec: secretory cells.
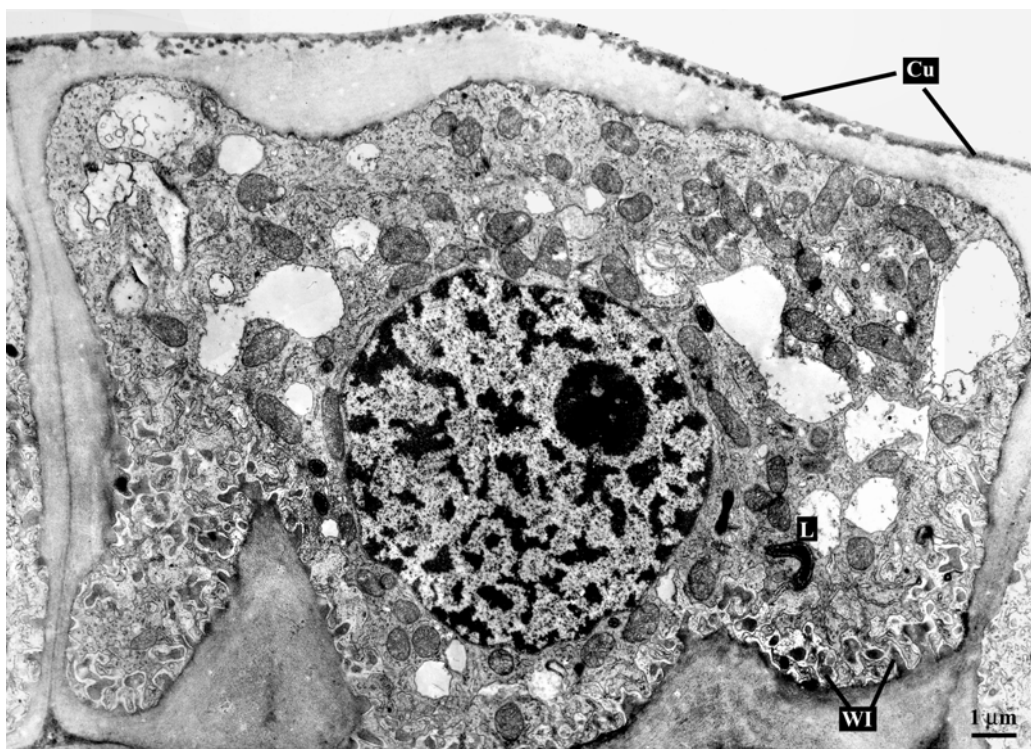

Fig. 2. General sectional view of the outer secretory cell of the digestive gland. Cu: cuticle; L: leucoplast; WI: wall ingrowths.

As shown in Tables 1-5 for the outer secretory cells, the parameters of the endomembrane system which include CCVs and their smooth derivatives designated together as protein vesicles (PVs) follow well-defined dynamics that are clearly related to the production of acid hydrolases.

In the cells of the meristematic glands the endoplasmic reticulum is poorly developed and the cytosol is a dominant cell component (Tables 1-3). Golgi stacks are fairly numerous (Table 4), as compared with the meristematic cells of other plants studied so far (Vassilyev and Muravnik, 1997; Vassilyev, 2000b). The frequency of the TGNs relative to that of Golgi stacks is low (1 : 3.6). Three types of secretory vesicles occur near TGNs (Fig. 3A) of which two types (CCVs and small, 50 nm, smooth vesicles) belong to PVs category and are infrequent (Table 3). These two types of vesicles are seen at all subsequent stages. The vesicles of the third, more frequent and larger type (Table 5) have granular-fibrillar contents (Fig. 3A) and they disappear from gland cells at the next stage. Morphometric data imply that at this stage the release of hydrolases into the gland cell walls does not yet occur and the number of PVs produced is sufficient to load only lytic vacuoles ensuring the lysosome function of the cells (Vassilyev, 1972, 2000b; Aubert *et al*., 1996). However, the main role of the Golgi apparatus in the gland cells at the meristematic stage is the synthesis of matrix polysaccharides of the cell walls and their secretion through exocytosis of granular-fibrillar vesicles.

At the second stage, *i.e.* the stage of the cell ingrowth deposition, large (Table 5) loosely-fibrillar vesicles are produced by Golgi stacks (Fig. 3B). They succeed the granular vesicles characteristic of meristematic glands. Their contents appear similar in structure to the wall ingrowths that are developing in the basal and the inner half of the anticlinal walls of secretory cells. These vesicles are apparently involved in the deposition of wall ingrowth material.

The RER greatly proliferates and the Golgi apparatus becomes highly active at the second stage (Tables 1-4). The surface density of the RER membranes per cell increases more than seven-fold and the number of PVs increases almost nine-fold. The frequencies of the Golgi stacks and TGNs also rise significantly, as well as their ratio (Table 4). More than half of the Golgi stacks are associated with the TGN. The diameter of the cisternae in stacks also increases (Table 5). These data strongly suggest a significant activation of hydrolase synthesis at this stage. The digestive enzymes are now deposited by means of CCVs and their smooth derivatives not only in the lytic vacuoles for storage but also in the secretory cell walls, the main site of their accumulation in the gland.

It is established that specific targeting signals are required for the transport of acid hydrolases from the TGN to lytic vacuoles (reviewed by Sarderfoot and Raikhel, 1999; Nebenführ and Staehelin, 2001; Neumann *et al*., 2003). By contrast, the dynamics of morphometrical data indicate that, like other secretory proteins (see review by Neumann *et al*., 2003), acid hydrolases in *D. lusitanicum* gland cells are transported in CCVs and their smooth derivatives to the plasma membrane as a default pathway for exocytosis, the standard process not requiring any targeting information.

In the secretory cells of fully mature glands the RER proliferates further (Tables 1, 2 and 5) and usually appears in the form of twisted narrow tubules (Fig. 4) instead of wider cisternae characteristic of previous stages. It is appropriate here to compare the *D. lusitanicum* gland with mammalian pancreas that also secretes digestive enzymes. The rate of secretory protein synthesis by the pancreatic exocrine cells is the highest among other known cell types (Case, 1978). One such cell in the rat contains RER with an average surface membrane density of

Table 1. Volume densities (%) of cell components in the outer secretory cells (means ± SE, n=15-23).

Gland state	Cell wall	Vacuole	Nucleus	Cytoplasm	Included in cytoplasm					
					Plastid	Mitochondria	Peroxisomes	Golgi stacks	RER	Cytosol
Stematic	10.6±0.8	14.7±2.2	17.2±3.0	57.5±3.5	1.9±0.4	4.2±0.5	0.06±0.02	0.8±0.2	1.7±0.2	48.8±2.2
Immature	21.9±1.4	40.1±3.4	5.6±1.2	31.9±3.2	1.6±0.2	4.3±0.4	0.07±0.01	1.3±0.1	2.5±0.2	22.1±2.3
Mature unstimulated	22.3±1.6	20.1±1.0	6.5±1.0	51.1±1.8	1.7±0.1	7.0±0.2	0.21±0.02	0.9±0.1	4.4±0.3	36.9±1.1
Stimulated for 1 h	22.7±1.0	16.1±1.4	6.4±1.4	54.8±3.0	1.9±0.2	6.0±0.5	0.30±0.03	2.4±0.2	8.5±0.2	35.7±1.9
Stimulated for 4 h	21.9±2.6	22.0±1.4	6.8±1.2	49.3±4.4	1.9±0.3	5.8±0.7	0.26±0.04	2.2±0.2	7.0±0.4	32.1±2.8
Stimulated for 8 h	22.6±1.8	20.9±1.4	6.6±1.2	49.9±4.4	1.5±0.2	8.2±0.7	0.12±0.06	2.5±0.4	9.3±1.2	28.3±1.9

Table 2. Secretory cell size and absorlute volumes of cell components in digestive glands (means and, for columns 2-4, means ± SE, n=15-21).

Gland state	Area[a]	Dimensions[b]		Volume[c]	Cell wall	Vacuoles	Nucleus	Cytoplasm	Components[d]					
		Anticlinal	Periclinal						Plastids	Mito-chondria	Included in cytoplasm			
											Peroxi-somes	Golgi stacks	RER	Cytosole
Meristematic	163.7±17.4	17.4±1.0	9.4±0.9	1540	163	226	265	886	29	65	1	12	26	753
Immature	287.9±33.3	17.3±0.5	16.5±1.8	4750	1040	1928	266	1516	76	204	3	62	119	1052
Mature unstimulated	405.3±15.8	18.1±0.4	22.4±1.0	9080	2025	1825	590	4640	154	635	17	82	400	3351
Stimulated for 1 h	426.6±21.9	18.8±0.3	22.2±0.9	9470	2151	1523	606	5190	180	568	28	226	808	3380
Stimulated for 4 h	410.1±43.6	18.4±1.4	21.8±1.1	8940	1957	1967	608	4408	170	518	23	197	626	2873
Stimulated for 8 h	405.8±4109	18.0±1.3	22.4±1.9	9090	2054	1900	600	4535	136	745	11	227	845	2572

a. Cell section area (μm²)
b. Cell dimentions (μm)
c. Cell volume (μm³)
d. Volume of cell components (μm³)

Table 3. Surface densities of RER membranes and frequencies of "protein" vesicles (means ± SE, n=16-32).

Gland state	RER membranes (μm²)		Number of vesicles						
	Cytosol[a]	Cell[b]	Cytosol[c]		Cytosol[a]		Cell[b]		All vesicles
			Near TGNs	Outside Golgi stacks	Near TGNs	Outside Golgi stacks	Near TGNs	Outside Golgi stacks	
Meristematic	8.6±0.6	650	0.2±0.06	1.0±0.2	2.8	19.5	200	1500	1700
Immature	43.9±3.9	4620	2.5±0.6	4.9±1.0	28.4	98.0	3300	11500	14800
Mature unstimulated	52.3±2.2	17520	0.8±0.04	1.8±0.07	9.8	39.1	3300	13000	16300
Stimulated for 1 h	98.6±6.8	33330	2.8±0.6	6.2±0.5	33.7	134.8	11400	45600	57000
Stimulated for 4 h	71.4±5.7	20510	1.8±0.3	5.1±0.6	21.7	110.9	6200	31900	38100
Stimulated for 8 h	95.2±7.5	24480	2.0±0.5	6.1±0.5	24.0	132.6	8200	45300	53500

a. Per 10 μm³ of cytosol
b. Per whole cell
c. Per 10 μm² of cytosol

Table 4. Organelle number (means ± SE, n=15-23).

Gland state	Plastids		Mitochondria		Peroxisomes		Golgi stacks		
	a	b	a	b	a	b	a	b	Including TGN[a]
Meristematic	7.4±1.2	68	18.9±2.2	193	0.4±0.1	4	5.13.6±0.90.6	36	1.43.6±0.90.3
Immature	3.2±0.5	28	31.2±3.3	545	0.8±0.08	15	8.53.6±0.91.1	81	4.53.6±0.90.5
Mature unstimulated	9.0±0.8	118	60.9±5.4	1124	2.3±0.3	45	9.03.6±0.90.5	103	2.73.6±0.90.2
Stimulated for 1 h	7.2±0.5	92	67.4±4.1	1631	4.6±0.5	134	18.93.6±0.91.4	178	9.53.6±0.90.8
Stimulated for 4 h	8.8±0.8	129	63.1±6.6	1562	4.7±1.0	123	14.43.6±0.91.6	118	5.63.6±0.90.6
Stimulated for 8 h	7.3±0.9	118	72.1±9.0	1615	3.6±0.9	142	15.43.6±0.91.6	123	7.43.6±0.91.1

a. Number per sectional profile of cell
b. Number per whole cell

Table 5. Parameters of Golgi stacks and secretory vesicles.

Gland state	Number of cisternae in a Golgi stack[a]				Diameters of Golgi stacks (μm)[b]			Diameters of vesicles (nm)[b]		
								"Protein" vesicles (nm)[b]		Others
	Cis	Medial	Trans	Total	Cis	Medial	Trans	Coated	Smooth	
Meristematic	1.6 (1-2)	2.0 (1-3)	2.2 (1-3)	5.0 (5-7)	0.50±0.03	0.84±0.06	0.66±0.04	73±3	49±3	106±7
Immature	1.3 (1-2)	1.7 (1-3)	2.2 (1-3)	5.1 (5-7)	0.70±0.04	0.94±0.03	0.89±0.04	88±6	51±4	253±9
Mature unstimulated	2.1 (1-3)	1.5 (1-2)	1.6 (1-3)	5.1 (5-7)	0.42±0.01	0.51±0.02	0.51±0.02	81±2	46±2	None
Stimulated for 1 h	1.2 (1-2)	2.1 (1-3)	2.8 (2-4)	6.1 (5-7)	0.64±0.04	0.89±0.05	0.89±0.03	83±2	50±1	None

a. means and, in parentheses, range of variation, n=15-19
b. means ± SE, n=15-36

6200 μm^2 (Cope, 1983) which represents a value of 2.8 times less than the secretory cell of the unstimulated digestive gland of *D. lusitanicum* (Table 3). Based on the established correlation between the amount of RER and the rate of protein synthesis (Uchiyama and Saito, 1982; Nevorotin, 1992) one can assume that the unstimulated secretory cell of *D. lusitanicum* may elaborate per unit of time more hydrolases than that of the rat pancreatic exocrine cell.

The volume density of Golgi stacks decreases 1.4-fold (Table 1), while their absolute volume per cell does not change (Table 2) in the mature stage. At the same time the number of stacks per cell increases 1.3-fold (Table 4). This apparent inconsistency is explained by the substantial decrease in the size of the Golgi stacks (Table 5) with their unchanged number per sectional view of cells (Table 4). The frequency of the TGNs with respect to that of the Golgi stacks decreases again to the value of 1 : 3.3 (Table 4). Large secretory vesicles disappear and may represent the cause of the size reduction of Golgi stacks. Only CCVs and their smooth derivatives are seen at the TGNs (Fig. 3C) and elsewhere in the cytoplasm (Fig. 3D). The concentration of these PVs situated both in the vicinities of the TGNs and outside the Golgi stacks falls almost three-fold. However, the total number of PVs per cell remains as high and, as in growing glands, the single smooth PVs situated outside Golgi stacks are predominant (Table 3). It can be inferred from the very large number of PVs and the increased number of stacks that not only the synthesis but also secretion into the cell walls of digestive enzymes continues in the mature glands at a high rate. Both processes proceed uninterruptedly, irrespective of whether or not an insect was caught. Thus, in unstimulated glands of *D. lusitanicum* the secretion appears to progress according to a constitutive type. Such a type of secretion is also characteristic of the mammalian pancreas (Uchiyama and Saito, 1982; Beaudoin and Grondin, 1991) in which exocrine cells of fasting animals continuously release hydrolases into the blood through their basolateral plasma membrane, rather than regulated secretion through the apical membrane that occurs in stimulated cells.

Within a few minutes after prey capture, the digestive fluid appears on the surface of the stimulated digestive glands of *D. lusitanicum* (Darwin, 1875; Fenner, 1904). Secretion continues and the digestive pool thus formed
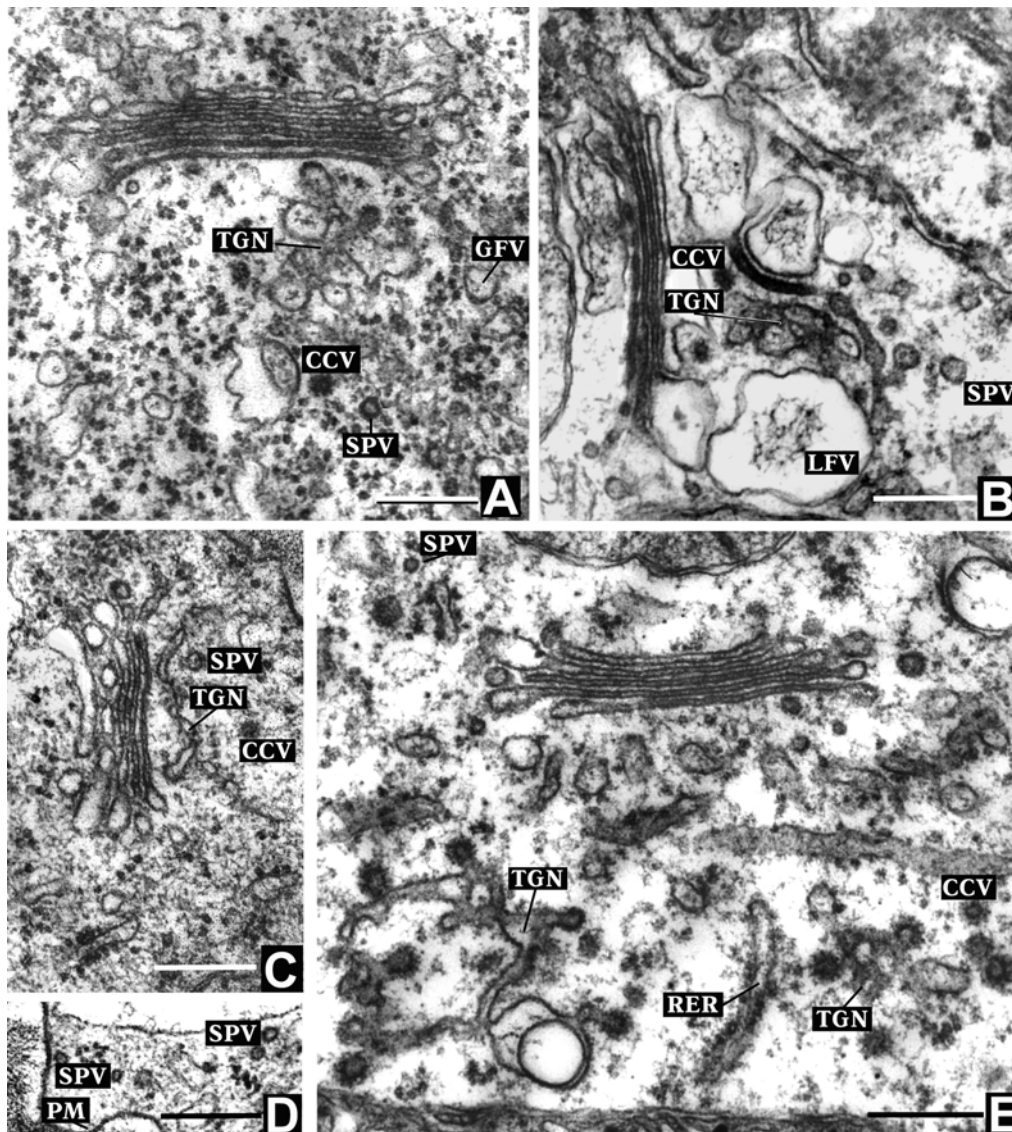
Fig. 3. Golgi stacks, *trans*-Golgi networks (TGNs) and secretory vesicles in the outer secretory cells of the digestive glands in their different states. A: Meristematic state. The TGNs are involved in the production of the granular-fibrillar vesicles (GFV) containing matrix polysaccharides and, to a lesser extent, clathrin-coated vesicles (CCV) loaded with acid hydrolases. SPV, smooth protein vesicle, a derivative of the CCV. B: Immature state. CCV loaded with hydrolases and loosely-fibrillar vesicles (LFV) loaded with pectin of the wall ingrowths are pinched off the TGN. C and D: Fully mature unstimulated state. No polysaccharide vesicles are produced by the TGN; the frequency of the clathrin-coated vesicles (CCV) and the smooth-protein vesicles (SPV) seen at the TGN are reduced, nevertheless SPV are scattered over the whole cytoplasm including the neighbourhood of the plasma membrane (PM). E: Stimulation for one hour. The diameter of cisternae in the Golgi stacks is increased and the larger number of the clathrin-coated vesicles (CCV) loaded with acid hydrolases is produced by the TGNs. Scale bars = 0.25 μm.

combined with the slime produced by the stalk glands drowns and digests the insect. The stimulation induces considerable changes in the quantitative ultrastructural parameters of gland secretory cells, especially the parameters of the endomembrane system. In glands stimulated for one hour, the volume density (Table 1) and absolute volume per cell (Table 2) of the RER as well as the surface densities of its membranes (Table 3) more than doubled.
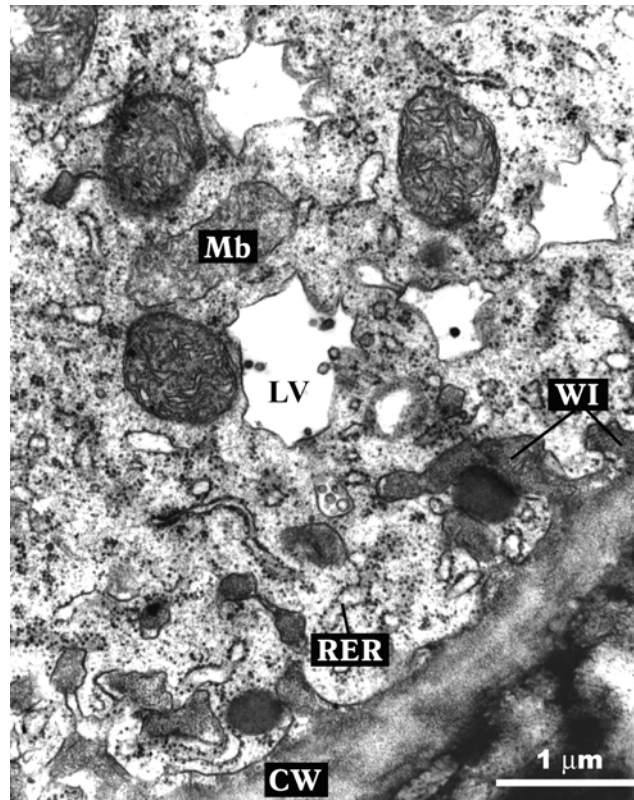
Fig. 4. Tubular rough endoplasmic reticulum (RER) and other organelles in the secretory cell of the mature unstimulated gland. CW: cell wall; LV: presumptive lytic vacuole; Mb: microbody; WI: wall ingrowths.

Volume density and absolute volume of the Golgi apparatus also increase. Both the number of Golgi stacks and especially TGNs rise, the former 1.7-fold, the latter 3.5-fold (Table 4). More than half of the stacks observed in sectional view are associated with the TGNs. The diameter of the cisternae in the Golgi stacks, particularly medial and *trans* ones, also rises (Table 5). These changes are accompanied by a dramatic rise in the activity of the Golgi apparatus in the PVs production, whose number per cell increases 3.5-fold (Table 3). Up to 5-7 CCVs may be seen forming in one sectional view of the TGN (Fig. 3E). Small vacuoles were occasionally seen with their tonoplast being in the direct contact with the plasma membrane (Fig. 5) suggesting subsequent anastomosis of membranes and exocytosis of vacuolar contents. Thus, the secretion of acid hydrolases from the lytic vacuoles into the gland cell walls by exocytosis appears to be a likely possibility. Structural evidence has been reported for the regulated exocytosis of lytic vacuoles in the digestive glands of *Dionaea muscipula* (Schwab *et al.*, 1969) and *Pinguicula vulgaris* (Vassilyev and Muravnik, 1988).

In contrast to vacuoles, PVs which number is exceptionally high in the gland cytoplasm are often seen in the immediate vicinity of the plasma membrane. These structural data indicate that the release of the digestive enzymes into the gland cell walls in *D. lusitanicum* after stimulation occurs mainly by way of default secretion of PVs. The discharge of acid hydrolases into gland cell walls through both exocytosis of PVs and lytic vacuoles is in agreement with the concept (Harsay and Schekmann, 2002; Linke *et al.*, 2002) that several branches of exocytic pathway may be used for the secretion of acid hydrolases including the movement of enzyme-loaded vesicles and vacuoles directly to the plasma membrane for exocytosis.
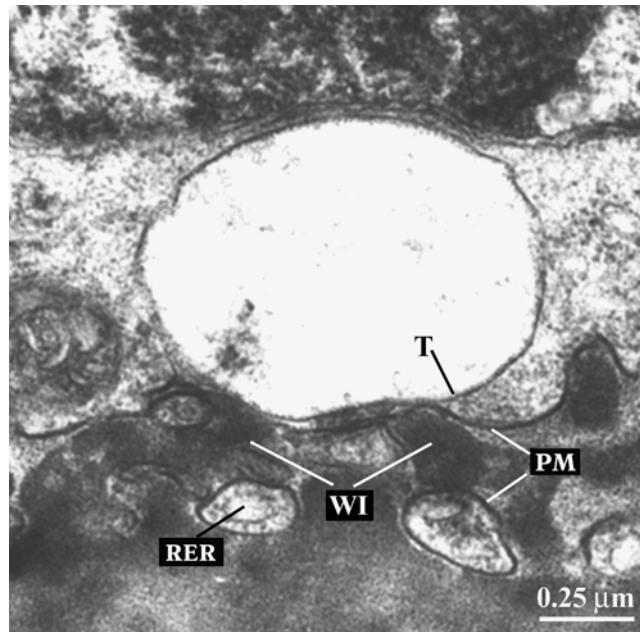
Fig. 5. Small, presumptive lytic, vacuole with the portion of its tonoplast (T) in direct contact with the plasma membrane (PM). RER: rough endoplasmic reticulum; WI: wall ingrowths.

The changes of morphometric parameters of endomembrane system in *D. lusitanicum* gland cells after stimulation cannot be interpreted otherwise but as implying a sharp rise in the rate of synthesis and secretion of digestive enzymes. Subsequent changes indicate some reduction in the rates of these processes, as is inferred from the Tables 3 and 4; nevertheless the rates remain much higher than in the cells of unstimulated glands.

The increase of the CCV frequencies in the vicinity of the TGNs upon stimulation was also noted in the secretory cells of digestive glands of *Pinguicula vulgaris* (Vassilyev and Muravnik, 1988), *Aldrovanda vesiculosa* (Muravnik *et al*., 1995; Muravnik, 1996), *Drosera anglica* and *D. rotundifolia* (Muravnik, 2000).

It is known that the capture of prey initiates an active transport of Cl^- and H^+ across the plasma membrane into the cell walls of the secretory cells of all carnivorous plants so far investigated including *D. lusitanicum* (Heslop-Harrison and Heslop-Harrison, 1980; Jung and Lüttge, 1980; Juniper *et al*., 1989). This secretion of ions induces, in that way, the flux of water through an osmotic gradient and the flush of acidic water carries hydrolases with it. The digestive fluid thus formed flows through the pores in the cuticle to the surface of the leaf and pours over the captured insect. The absorption of breakdown products occurs by the same glands that release the digestive fluid (Juniper *et al*., 1989).

Based on the results obtained in this study and the available information cited above the following hypothetical models of digestive gland functioning may be suggested (Figs. 6A-C). There are two current views on the mode of cargo and membrane transport through the Golgi stacks, namely, the "cisternal progression-maturation model" and the "shuttle vesicle transport between stationary cisternae in the Golgi stack model" (Andreeva *et al*., 1998; Nebenführ and Staehelin, 2001; Neumann *et al*., 2003). Provisionally, the former was used in this study for meristematic gland cells that are mainly involved in cell wall polysaccharide secretion (Fig. 6A), whereas the shuttle vesicle model was employed for hydrolase processing (Figs. 6B and C). The models of *D. lusitanicum* gland functioning proposed here also imply a

Meristematic gland

Mature, before prey capture
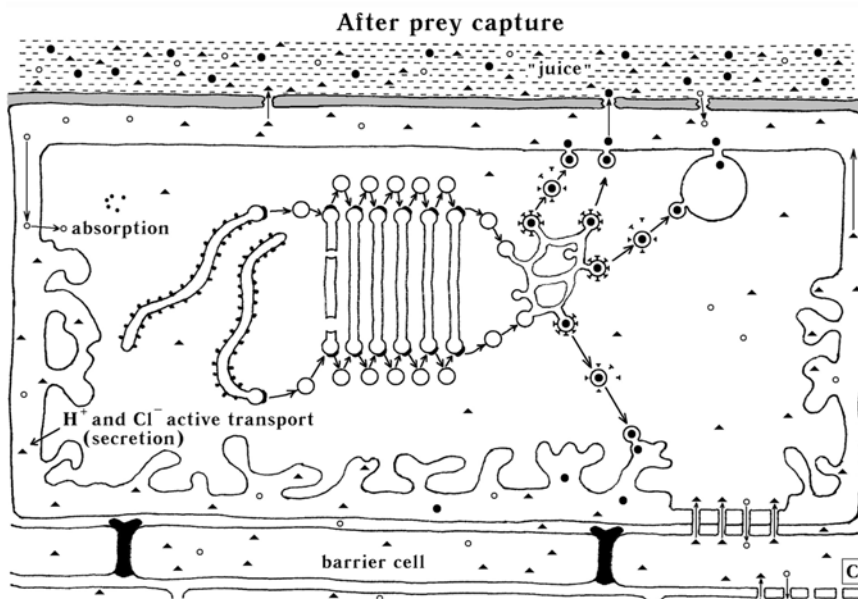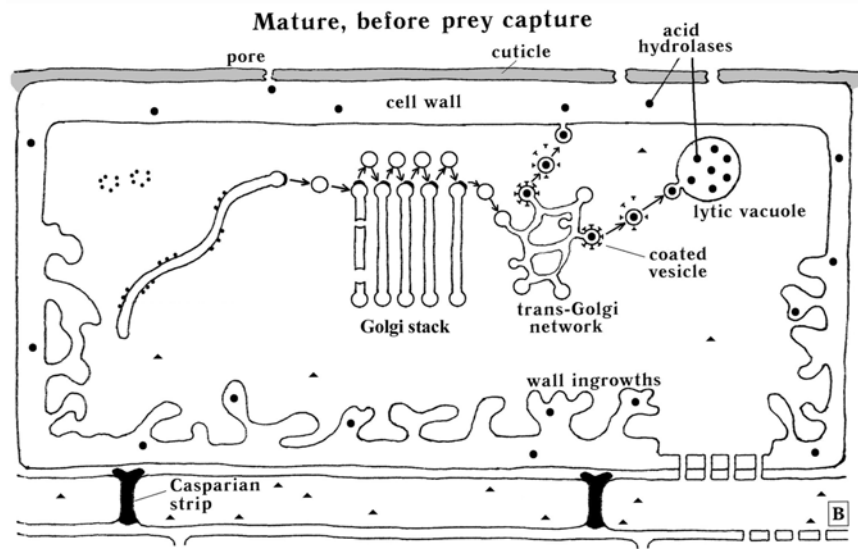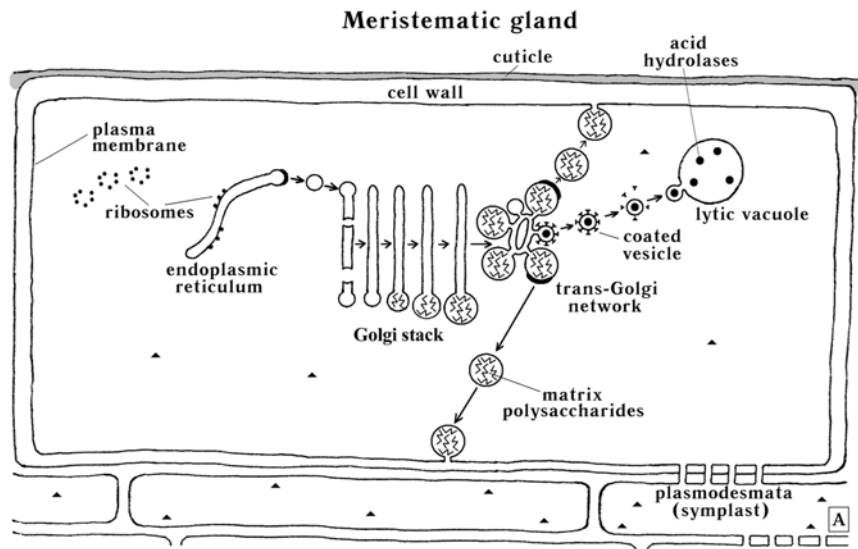

After prey capture

Fig. 6. Hypothetical models of the digestive gland cell functioning in *Drosophyllum lusitanicum* based on the evidence from ultrastructural morphometry. Only a cell of the outer tier of secretory cells is shown. A: Meristematic state. B: Mature state before prey capture. C: Mature state after prey capture. In the meristematic state, the Golgi apparatus is mainly involved in the synthesis and secretion of matrix polysaccharides of the cell walls (arrows indicate a cisternal progression from cis- to trans-faces of a Golgi stack according to the cisternal progression model). In all states of glands acid hydrolases are synthesized at the rough endoplasmic reticulum (RER) membrane bound ribosomes. They are co-translationally inserted into the RER lumen. Thereafter they are transferred via special type of vesicles into the cis-cisterna of Golgi stacks. After processing through Golgi cisternae mediated by another special type of transport vesicles, proteins enter the trans-Golgi network (arrows indicate the transport vesicle movement between stationary cisternae of a Golgi stack). In the trans-Golgi network, acid hydrolases (closed circles) are segregated and concentrated in clathrin-coated vesicles. Soon after the detachment of the vesicles from the trans-Golgi networks they lose their coat and their smooth derivatives transfer their cargo to the lytic vacuoles (state A) or to both vacuoles and plasma membrane (states B and C). After the fusion of the vesicle membrane with the plasma membrane (secretion by means of exocytosis), the acid hydrolases are found in the cell walls. Hydrolase secretion starts in the immature glands and continues in mature ones irrespective of prey capture (constitutive secretion). In unstimulated glands (B) their surface remains practically dry, and hydrolases are stored within the cell walls and wall ingrowths. The prey capture induces intensification of the hydrolase synthesis (shown here by increased number of rough endoplasmic reticulum cisternae and frequencies of membrane bound ribosomes) and (shown by increased diameter and number of Golgi cisternae and increased frequencies of transport vesicles and PVs) as well as the exocytosis of some lytic vacuoles (regulated secretion). Digestive juice appears on the surface of the leaf (C). The active transport of chloride ions and protons (closed triangles) across the plasma membrane out of the cytoplasm drives water into the cell walls along an osmotic gradient. Due to the wall ingrowths, the surface area of the plasma membrane, which is the site of the ionic pumps, is greatly increased. Acidic water carries hydrolases with it through pores in the cuticle and onto the gland surface. Casparian strips of the barrier cells block the flow of ions from the walls of the secretory cells back into the leaf. The soluble products of digestion (open circles) are absorbed through the plasma membrane of secretory cells. They then move through plasmodesmata into the second layer (not shown here) of secretory cells and next into barrier cells and farther into the leaf tissues. Only anterograde movement of transport vesicles through Golgi stack is shown in the diagrams.

constitutive (spontaneous) secretion of hydrolases in immature and mature unstimulated glands via PVs (Figs. 6A and B) and both constitutive and regulated (stimulated) secretion via lytic vacuoles (Fig. 6C) in stimulated glands.

CONCLUSION

The morphometric data obtained in the present study indicate that there is a continuous acid hydrolase secretion in the immature and mature unstimulated digestive glands of *Drosophyllum lusitanicum*. The secretion is mediated by CCVs. Both the rate of the synthesis and secretion increase further on stimulation. Fundamentally different subcellular strategy of adaptation to carnivory appears to operate in *Aldrovanda vesiculosa* and *Drosera rotundifolia*. As the electron microscope evidence implies (Muravnik *et al.*, 1995; Muravnik, 1996, 2000), there is no acid hydrolase synthesis in these plants prior to stimulation. However, the protein synthetic apparatus, RER, is formed in the digestive glands at the early stage of leaf growth but it assumes an inactive state and appears as stacks of parallel cisternae with the membrane bound ribosomes in the form of monosomes. Only a few TGNs are formed that practically lack CCVs. No sooner had the prey was captured than the protein synthetic apparatus is activated, the RER stacks are disassembled into the individual cisternae, the ribosomes are aggregated into polysomes and the TGNs produce numerous CCVs These ultrastructural changes imply the onset of hyhrolase synthesis and secretion, according to regulated type.

It is interesting to note that these two strategies of the digestive gland cell functioning are basically analogous to the two subcellular strategies characteristic of the pancreas of mammals on the one hand and that of amphibia and reptiles on the other (Case, 1978).

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LITERATURE CITED

Andreeva, A., M. A. Kutuzov, D. E. Evans and C. R. Hawes. 1998. The structure and function of the Golgi apparatus: a hundred years of questions. J. Exp. Bot. **49**: 1281-1291.

Aubert, S., E. Gout, R. Bligny, D. Marty-Mazars, F. Barrieu, J. Alabouvette, F. Marty and R. Douce. 1996. Ultrastructural and biochemical characterization of autophagy in higher plant cells subjected to carbon deprivation: control by the supply of mitochondria with respiratory substrates. J. Cell Biol. **133**: 1251-1263.

Beaudoin, A. R. and G. Grondin. 1991. Secretory pathways in animal cells: with emphasis on pancreatic acinar cells. J. Electron Microsc. Techn. **17**: 51-69.

Beevers, L. 1996. Clathrin-coated vesicles in plants. Intern. Rev. Cytol. **167**: 1-35.

Case, R. M. 1978. Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. Biol. Rev. **53**: 211-233.

Coleman, J., D. Evans, C. Hawes, D. Horsley and L. Cole. 1987. Structure and molecular organization of higher plant coated vesicles. J. Cell Sci. **88**: 35-45.

Cope, G. H. 1983. Exocrine glands and protein secretion: a stereological viewpoint. J. Microscopy. **131**: 187-202.

Darwin, C. 1875. Insectivorous plants. John Murray, London, UK. 462 pp.

Fenner, C. A. 1904. Beiträge zur Kenntnis der Anatomie, Entwicklungsgeschichte und Biologie der Laubblätter und Drüsen einiger Insectivoren. Flora **94**: 1-104.

Harsay, E. and R. Schekman. 2002. A subset of yeast vacuolar protein sorting mutants is blocked in one branch of the exocytic pathway. J. Cell Biol. **156**: 271-285.

Heslop-Harrison, Y. 1975. Enzyme release in carnivorous plants. In: Dingle, J. T. and R. T. Dean (eds.). Lysosomes in Biology and Pathology, **4**: 525-576. North Holland Publ. Co: Amsterdam, the Netherlands.

Heslop-Harrison, Y. and J. Heslop-Harrison. 1980. Chloride ion movement and enzyme secretion from the digestive glands of *Pinguicula*. Ann. Bot. **45**: 729-731.

Higashi, S., A. Nakashima, H. Ozaki, M. Abe and T. Ushiumi. 1993. Analysis of feeding mechanism in a pitcher of *Nepenthes* hybrida. J. Plant Res. **106**: 47-54.

Hillmer, S., A. Movafeghi, D. G. Robinson and G. Hinz. 2001. Vacuolar storage proteins are sorted in the *cis*-cisternae of the pea cotyledon Golgi apparatus. J. Cell Biol. **152**: 41-50.

Jung, K. D. and U. Lüttge. 1980. Effects of fusicoccin and abscisic acid on sugar and ion transport from plant glands. Ann. Bot. **45**: 339-349.

Juniper, B. E., R.J. Robins and D. M Joel. 1989. The Carnivorous Plants, Academic Press, London, UK. 353 pp.

Le Borgne, R. and B. Hoflack. 1998. Protein transport from the secretory to the endocytic pathway in mammalian cells. Biochim. Biophys. Acta, 4 Ser., **1404**: 195-209.

Linke, M., V. Herzog and K. Brix. 2002. Trafficking of lysosomal cathepsin B-green fluorescent protein to the surface of thyroid epithelial cells involves the endosomal/ lysosomal compartment. J. Cell Sci. **115**: 4877-4889.

Muravnik, L. E. 1996. Morphometrical approach to the secretory activity determination in digestive glands of *Aldrovanda vesiculosa* (Droseraceae). Bot. Zhurnal (Russia), **81**: 1-8. (In Russian, with English summary)

Muravnik, L. E. 2000. The effect of chemical stimulation on the ultrastructure of secretory cells of glandular hairs in the two species of *Drosera*. Russian J. Plant Physiol. **47**: 614-623. (In Russian)

Muravnik, L. E., A. E. Vassilyev and Y. Y. Potapova. 1995. Ultrastructural aspects of digestive gland functioning in *Aldrovanda vesiculosa*. Russian J. Plant Physiol. **42**: 5-13. (In Russian)

Nebenführ, A. and L. A. Staehelin. 2001. Mobile factories: Golgi dynamics in plant cells. Trends Plant Sci. **6**: 160-167.

Neuhaus, J. M. and J. C. Rogers.1998. Sorting of proteins to vacuoles in plant cells. Plant Mol. Biol. **38**: 127-144.

Neumann, U., F. Brandizzi and C. Hawes. 2003. Protein transport in plant cells: in and out of the Golgi. Ann. Bot. **92**: 167-180.

Nevorotin, A. J. 1992. Endoplasmic reticulum, a review. Tsitologia (Russia) **34**: 8-16. (In Russian, with English summary)

Robinson, D. G., G. Hinz and S. E. H. Holstein. 1998. The molecular characterization of transport vesicles. Plant Mol. Biol. **38**: 49-76.

Sarderfoot, A. A. and N. Raikhel. 1999. The specificity of vesicle trafficking: coat proteins and SNAREs. Plant Cell **11**: 629-641.

Scales, S. J., M. Gomez and T. E. Kreis. 2000. Coat proteins regulating membrane traffic. Intern. Rev. Cytol. **195**: 67-144.

Schmid, S. L. 1997. Clathrin-coated vesicle formation and protein sorting: an integrated process. Ann. Rev. Biochem. **66**: 511-548.

Schnepf, E. 1960. Zur Feinstruktur der Drüsen von *Drosophyllum lusitanicum. Planta* **54**: 641-674.

Schnepf, E. 1961. Licht- und elektronenmikroskopische Beobachtungen an Insektivoren-Drüsen über der Sekretion des Fangschleim. Flora **151**: 73-87.

Schwab, D. W., E. Simmons and J. Scala. 1969. Fine structure changes during function of the digestive gland of Venus's-flytrap. Amer. J. Bot. **56**: 88-100.

Staehelin, L. A., T. H. Giddings, J. Z. Kiss and F. D. Sack. 1990. Macromolecular differentiation of Golgi stacks in root tips of *Arabidopsis* and *Nicotiana* seedlings as visualized in high pressure frozen and freeze substituted samples. Protoplasma **157**: 75-91.

Steer, M. W. 1981. Understanding Cell Structure, Cambridge University Press, Cambridge, UK. 126 pp.

Uchiyama, Y. and K. Saito. 1982. A morphometric study of 24-hour variations in subcellular structures of the rat pancreatic acinar cells. Cell Tissue Res. **226**: 609-620.

Vassilyev, A. E. 1972. The problems of endocytosis and autophagy in a plant cell. In: Danilova, M. F. and G. M. Kozubov (eds.). Ultrastructure of Plant Cells. pp. 3-60. Nauka Press, Leningrad, Russia. (In Russian, with English summary)

Vassilyev, A.E. 1977. Functional Morphology of Plant Secretory cells. Nauka Press, Leningrad, Russia. 206 pp. (In Russian)

Vassilyev, A. E. 2000a. Quantitative ultrastructural data of secretory duct epithelial cells in *Rhus toxicodendron*. Intern. J. Plant Sci. **161**: 615-630.

Vassilyev, A. E. 2000b. Cell component dynamics in the leaf tissues of *Arabidopsis thaliana* (Brassicaceae) during differentiation. 1. The apical meristem and leaf primordia. Bot. Zhurnal (Russia) **85**: 68-83. (In Russian, with English summary)

Vassilyev, A. E. 2002. Functional ultrastructure of *Drosophyllum lusitanicum* digestive glands. 1. Secretory cells before stimulation. Bot. Zhurnal (Russia) **87**: 1-12. (In Russian, with English summary)

Vassilyev, A. E. and L. E. Muravnik. 1988. The ultrastructure of the digestive glands in *Pinguicula vulgaris* (Lentibulariaceae) relative to their function. II. The changes on stimulation. Ann. Bot. **61**: 343-351.

Vassilyev, A. E. and L. E. Muravnik. 1997. Cell component dynamics in the leaf tissues of *Populus deltoides* (Salicaceae) during the life cycle. 1. Shoot apical meristem. Bot. Zhurnal (Russia) **82**: 1-17. (In Russian, with English summary)

Vitale, A. and G. Galili. 2001. The endomembrane system and the problem of protein sorting. Plant Physiol. **125**: 115-118.

露葉毛氈苔之消化性分泌毛茸在成熟過程與受刺激後微細構造的動態變化

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摘　　　要

　　本研究針對露葉毛氈苔 *Drosophyllum lusitanicum* Link (Droseraceae)分泌酸性水解酶之消化性毛茸的分泌細胞的內膜系統加以量化的探討。分泌毛茸之細胞的粗內質網膜系表面的密度，以及高基氏體、clathrin 被膜小囊胞 (CCVs)與其衍生的平滑小囊胞的個數，均隨著分泌毛茸的成熟而逐漸增加，在完全成熟而未刺激之毛茸的每一分泌細胞，含有之粗內質網膜系的平均表面積為 17500 μm^2 以及 16000 個小囊胞（包括平均直徑為 81 nm 的CCVs與其衍生成之平均直徑為 46 nm的小囊胞）。在捕捉模擬餌食之刺激後一小時，粗內質網膜系表面的密度、高基氏體的個數、以及蛋白質小囊胞均大量增加。實驗數據顯示由CCVs衍生之平滑小囊胞的形成即意味著分解酶的分泌。分泌毛茸在未成熟時即開始分泌水解酶，且在成熟時期不論其是否確已捕捉到昆蟲均繼續分泌。藉由刺激，消化性毛茸合成的水解酶與其分泌的速率均明顯提高，並造成葉面上消化性液體的釋出。除了蛋白質小囊胞，尚會產生其他兩類之較大的高基氏體分泌小囊胞：顆粒纖維狀者（直徑大約 105 nm，其侷限在分生時期的細胞）以及疏鬆纖維狀者（直徑大約 250 nm，僅形成於細胞壁向內生成堆積的時期）。本文提出模式以說明消化性毛茸分泌細胞在刺激之前與刺激之後的功能。

關鍵詞：酸性水解酶之分泌、食蟲植物、被膜小囊胞、*Drosophyllum*、形態計量、微細構造。

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