Tree and Forestry Science and Biotechnology ©2008 Global Science Books

Ultrastructural Study of Transcytosis in Parenchymal Tissues of *Ginkgo biloba*

Yuping Ling* • Li Li • Lili Wang • Bin Pan • Bo Wang*** • Peng Chen******

School of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China

Corresponding authors: *** lyplxj@yahoo.com.cn ** lili225009@hotmail.com *** wangbodzy@yahoo.com.cn **** chenpeng@yzu.edu.cn**

ABSTRACT

Transcytosis was examined in the parenchymal cells of the vegetative tissues of *Ginkgo biloba* with electronic microscopy, with the following results. (1) There are two possible modes of transcytosis based on morphological characteristics, involving either the transportation of pit fields or endocytosis and exocytosis. No plasmodesmata were observed in the parenchyma of any vegetative ginkgo tissue. (2) Pit fields in the cell walls of the parenchyma are simple symmetric pits, with 3–5 usually joined together like beads. The thickness of the adjacent cell walls is about 1 μm, and the diameter of the narrowest pit field is 0.2–0.3 μm. Cellular organelles congregate near the pit. (3) There is a great deal of endocytotic and exocytotic activity in the parenchymal cells. There are at least two forms of endocytosis: receptor-mediated endocytosis and fluid-phase endocytosis, in which ellipsoid or spherical microcysts with diameters of approximately 0.05–0.1 μm, separately imbibe the large macromolecular liquid-phase substances dissolved in the matrix. (4) When endocytosis and exocytosis begin, the part of the plasma membrane next to the cell wall sinks, forming a ligand-coated pit, afterwards wrapping the receptor within the caveola. The plasma membrane near the cell wall adheres to it and separates from the plasma membrane to form coated vesicles, which then enter the cell. Finally, the ligand partially or entirely decomposes, and the caveola enters the cell via a lysosome. (5) In mesophyll cells, the plasma membrane extends gemma-like extrusions after it sinks, part of which form sub-gemma. These separate from the venter-gemma to enter the cytosol. In endocytosis, there are two types of sub-gemma vesicles: the electron density of one is low, similar to that of the liquid phase, and the other has a rich fibrous stripe structure.

Keywords: endocytosis and exocytosis, gemma-like extension, ultrastructure
Abbreviations: GPI, glycosyl-phosphatidylinositol

INTRODUCTION

In the middle of the 1970s, after Steinman *et al*. first discovered the system of plasma membrane recycling and Goldstein *et al*. completely described the receptor-mediated endocytosis of low-density lipoproteins, endocytosis became a hotspot of research within the discipline of cytobiology. Research has shown that endocytosis is the essential way in which nearly all true nucleated cells absorb macromolecular matter from the extracellular matrix. Many macromolecules are involved, including hormones, growth factors, and transport proteins, which carry nutrients or regulatory signals, lysosomal enzymes, immunoglobulins, viral toxins, and macromolecular organic matter (Willingham and Pastan 1984), which synthesize manually. However, with electron microscopy, the Japanese scholar Yamada (1955) first observed the caveolae, which exist in the plasma membrane. These were later confirmed to be the actual cellular organ that transports some macromolecules in the process of endocytosis. Anderson (1991) used an electron microscope to discern the characteristic shape of the caveolae, observing a fibrous stripe in each caveola, and reporting that sometimes many caveolae are connected into strings. Anderson proposed that the caveolae not only transport macromolecular matter into the cell but also perform the physiological function of transmitting certain information.

The ginkgo is a unique gymnosperm in China and was extremely abundant in the Jurassic Period of the Mesozoic era, the "golden age" of the ginkgo, when there were at least 14 species. From the last stages of the Tertiary period to the early Pleistocene, many ginkgo plants had already become extinct as a result of the prevailing glaciation. Only one species remains extant in our country and this prehistoric gymnosperm has a rich and unique character in both botanical and molecular biological terms. There has been much research into endocytosis and exocytosis in the medical domain (Caldwell and Slapnick 1992; Smart *et al.* 1995). However, endocytosis and exocytosis in the gymnosperms has not been reported until now. In this study, we investigated some of the cytobiological characteristics of this tree and its vegetative organs. We report here these tissues and mechanisms of ginkgo, initially at the cellular level.

MATERIALS AND METHODS

Mature fruit of the variety "MaLing" were picked from large adult *Ginkgo biloba* trees at the Jiangyan Fruit Tree Testing Facility, in Jiangsu Province. These were then stratified at low temperature, and the seeds were sown into a matrix of pure perlite. The seedlings were raised in an illuminated temperature-controlled incubator, at 25°C with sunlight for 8 h per day, for about 20 days. When the seedlings were about 3–5 cm high, with 2–3 young leaves, we began to take samples. Samples of roots consisted of root sections, about 2 mm long, taken from the root hair area, to within about 0.5 cm of the root point. The stem samples consisted of 2 mm stem sections taken to within about 1 cm of the stem point. The materials were fixed in 3% glutaric dialdehyde fixer for 3 h, then washed three times with buffer solution (pH 7.0), and fixed with 1% perosmic acid for 3 h. The samples were dehydrated in a graded series of ethyl alcohol, treated with epoxypropane, embedded in Epon812, and dried in a drying oven at different temperatures (35, 45, 60°C) for 24 h. Ultrathin sections were cut, and these were observed and photographed with an electron microscope.

Received: 2 February, 2008. Accepted: 17 June, 2008.

Original Research Paper

RESULTS AND ANALYSIS

Ultrastructural observations of transportation in pit fields

When the sections were observed with an electron microscope, we discovered that the primary pit fields (**Fig. 1**) in the parenchymal cells of vegetative tissues occur singly, in pairs, or as 3–5 pits tightly joined in a bead-like form. Whereas the other parts of the plasma membrane appear thick, with a diameter of 1–1.5 μm, the plasma membrane containing the pits is thin, with a diameter of only 0.2–0.3 μm. The pits form channels between the cells. This kind pit does not have a secondary wall and its structure is simple; it is a simple symmetric pit. The cellular organelles are clustered relatively dense in the pit, especially mitochondria, some microbodies, and starch grains. The pit field is the primary locus and channel for the transportation of materials and for cell signaling. In the parenchymal cells of the vegetative tissues of ginkgo, these cellular organelles may be necessary for both transportation and signaling. Thus, transportation across the cells is active transport and therefore requires energy. Starch and chondriosomes are the sources and donors of this energy. However, after observation of many sections of vegetative tissues, including root, stem, and leaf tissues, we report that no plasmodesmata are present in these tissues. In most plants, plasmodesmata exist in the cell walls of parenchymal cells, especially in the pit field, and these channels are completely responsible for the transportation of materials and the cell signaling of these cells.

Observation of receptor-mediated endocytosis in the parenchymal cells of ginkgo vegetative tissues

In these cell sections, we observed that at the beginning of receptor-mediated endocytosis, part of the plasma membrane near the cell wall forms a receptor (**Fig. 2**) by invagination, thus forming coated pits. Caveolae and the materials for ligand-mediated endocytosis gradually move to the center of the coated pit. The coated pits are ellipsoid or round, and differ in size. Their diameters are 50–90 nm and their electron density is high. They have a dispersed distribution in the coated pits (**Fig. 3**). The coated pit, in which the receptors and ligands are combined, undergoes further invagination. Two langmuir, which are on the nonplasma membrane, begin to fuse (**Fig. 4**). With fusion, the plasma membrane gradually fractures and detaches from the cell plasma membrane to form an early endosome from the coated vesicles before it enters the cell (**Fig. 5**). The early endosome, which is free in the protoplasm, gradually becomes a late endosome. The late endosome is dissolved by proteolytic enzymes. The structure of some membranes begins to fracture (**Fig. 6**). The caveolae are released from the endosome and move into the protoplasm, to complete the endocytosis (**Fig. 7**). Further observations showed that there are two types of caveolae, which are free in protoplasm. The most common are all ellipsoid, and have a signal membrane and a complex internal structure. These caveolae have a complex intima system, like the cristae of the chondriosome, with other electron-brilliant clear areas (**Fig. 8**). The other type of caveola has single membranes that encase corpuscles. The corpuscles are highly electron dense and are joined together. There is a highly electron-dense material in the center of the corpuscle, like a nucleus (**Fig. 9**). Through ultrastructural observations, we have confirmed that the caveolae constitute real cellular organs, as suggested by Anderson (1991). When it was recognized that caveolae have a variety of structures, it was proposed by Fujimoto *et al.* (1992) and Travis (1993) that this validated, to a certain extent, the different physiological functions of the caveolae.

Observation of gemma-like extensions and liquid-phase endocytosis in ginkgo vegetative parenchymal tissues

We observed gemma-like extensions in liquid-phase endocytosis, especially in young leaf tissues. After the gemma-like extensions are produced, the plasma membrane fuses to form baggy or cystoid venter-gemma (**Fig. 10**), containing an anomalous substance. The plasma membrane of the venter-gemma then begins to form one or more sub-gemma (**Fig. 11**). The sub-gemma gradually grows and matures. When it is close to the signal membrane, which is connected to the main gemma, it begins to fuse and detaches from the venter to form small vesicles, which are free in the protoplasm. At this time, endocytosis is complete (**Fig. 11**). After the mature sub-gemma detaches from the venter-gemma, a new sub-gemma is formed on the venter-gemma. The venter-gemma does not detach from the plasma membrane, but stays on the plasma membrane for a long time. A micrograph shows this substance and that the small vesicles have single membranes. This kind of gemma is regularly spherecal or irregular, and contains a substance that is a brilliantly clear liquid, which is free in the protoplasm after endocytosis. The region adjacent to the cell protoplasm is electron dense. Another small vesicle had a structure with abundant nemaline stripes (**Fig. 12**).

DISCUSSION

The cell is an open system, in which all the activities of life occur, so there must be an exchange of matter between the cells themselves and with the environment. There are two modes of transportation across cells: symplastic transport and transmembrane transport. Symplastic transport always involves the plasmodesmata between neighboring cells, which can transport most of the matter in the cells, including water, mineral nutrients, and signals (Robards and Lucas 1990; Yu *et al.* 1998). The transportation of organic nutrients inside the cell also occurs through the plasmodesmata. Research has shown that even the karyon can move through the plasmodesmata (Guo *et al.* 1990; Ding *et al.* 1999). However, according to the results of this study, there

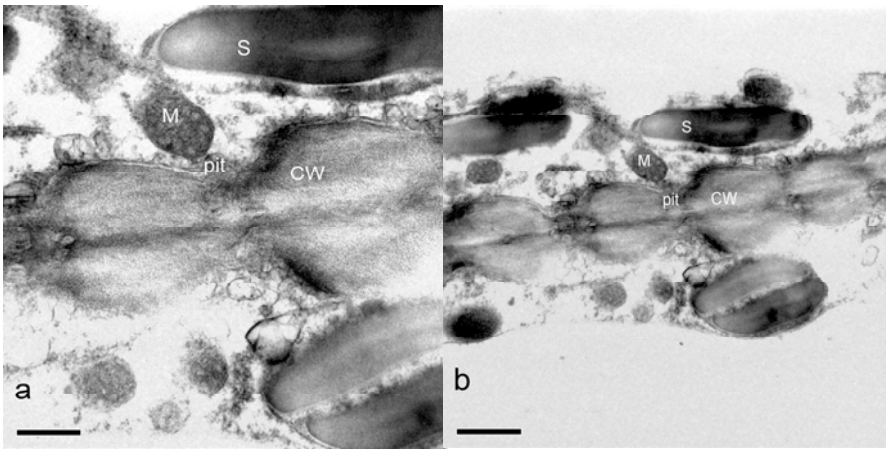
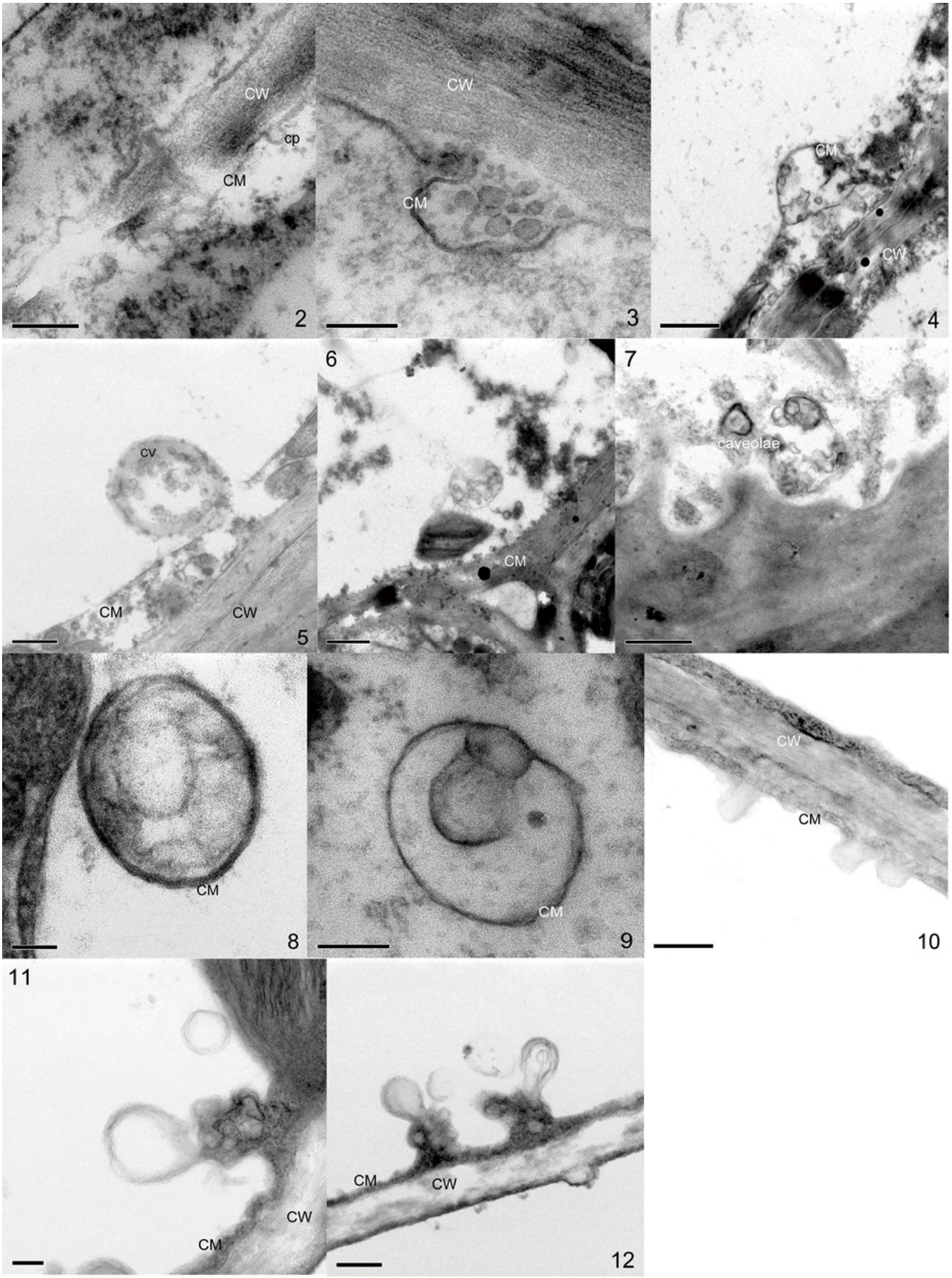


Fig. 1 Channels in parenchymal cells of the ginkgo apical stem. (**a**) One primary pit field, CW = cell wall, M = mitochondria, S = starch, (Bar = 0.5 μm); (**b**) a group of primary pit fields (Bar = 1 μm).



Figs 2-12. (**2**) Plasma membrane begins to sink and to form a coated pit, CM = cell membrane, cp = coated pit. Bar = 200 nm. (**3**) Caveola begins to assemble and to form a coated pit. Bar = 200 nm. (**4**) The nonplasma membrane begins to fuse. Bar = 1 µm. (**5**) The plasma membrane gradually fractures after fusion. cv = coated vesicle. Bar = 0.5 µm. (**6**) The structures of some membranes begin to fracture. Bar = 1 µm. (**7**) A caveola is released from the endosome. Bar = 1 µm. (**8**) One type of caveola has an abundant intima system. Bar = 100 nm. (**9**) Highly electron-dense material in the center of the corpuscle, like a nucleus. Bar = 200 nm. (**10**) Plasma membrane fuses to form a baggy or cystoid venter-gemma. Bar = 0.5 µm. (**11**) Endocytosis is complete. Bar = 0.2 µm. (**12**) Small vesicle has a structure with abundant nemaline stripes. Bar = 0.5 µm.

are no plasmodesmata in the parenchymal cells of the vegetative tissues of ginkgo. We only observed areas of cellulose containing single pit fields, with no plasmodesmata in the ultrastructure of the pit fields, which transgresses classical botanical theory. Whether there are no plasmodesmata in the parenchymal tissues of ginkgo, or our observations are artifacts of the sampling process, sample treatment, electron microscopy, or our experimental technique, this study must be repeated to confirm our conclusions. In these experiments, we also observed that many organelles, especially mitochondria, are distributed around the pits field, which indicates that the pit field is an active metabolic area. Further chemical analysis of the H^+-ATPase enzyme in this transmembrane region will confirm the function of the pit field in transport between cells.

There are many ways to study endocytosis and classical morphological observation is always effective. Using chemical technology to trace cells with electron microscopy or light microscopy allows us to observe the progress of the ligand undergoing endocytosis into the cell after it has bound its receptor in the plasma membrane, or as it moves between cells. Depending upon the mode of endocytosis, researchers often use receptor-mediated endocytosis tracers, such as low-density lipoprotein receptors, adsorptive endocytosis tracers, such as some lectins and cationic ferritin, and liquid-phase endocytosis tracers, such as horseradish peroxidase. Furthermore, biological and chemical methods have been used to study the progress of endocytosis. With the popularization of molecular biology technology, the components of the membrane vesicles have been analyzed. This research has also penetrated to the molecular level, involving the cloning of genes, the purification of receptor proteins, changing the receptor for gene heterogeny, and the study of the endocytosis of some membrane proteins. There have been many studies of endocytosis (Ouyang and Xie 1996). In this study, we primarily confirmed that the transmembrane in the parenchyma of *G. biloba* functions as plasma membrane vesicles in endocytosis to transport at least liquids. We observed that the receptor was derived and the ligand entered the protoplasm. However, further study is required to establish whether the endocytosis of the receptor and ligand is recirculated or derived. Our results indicate that the ligand caveola has the character of a cellular organelle. The emphasis of our future study will be the other roles of endocytosis in the mitosis, tissue development, etc., in ginkgo.

During the process of endocytosis in the cells of the parenchymal tissues of the leaf, we observed that the caveolae had obvious fibrous stripes. This is consistent with the results of Anderson, who investigated the permeability of the rat lung in the early 1990s. Lisanti thought that the caveolae were a major component of signal transmission, and could internalize the signals of hormones, growth factors, and extracellular regulators. Many receptors on the surface of the cell, such as the β-adrenergic receptor, bacterial toxins modified by the G-protein, the M-acetylcholine receptor, and chromium trichloride, all occur in caveolae. However, for now, the transmission of signals by the caveolae is not supported by direct experimental evidence. Lisantl *et al.* (1995) considered that signals outside the cell enter the microregion of the caveolae through glycosylphosphatidylinositol (GPI)–ankyrin on the plasma membrane. With the help of out space of membrane lipid bilayer of two lipid unsaturation of GPI–ankyrin, could the signal molecule recognizes GPI–ankyrin and reacts with it. Meanwhile, the caveolae react with the G-protein or tyrosine kinase, after which the signal outside the cell is transmitted into the cell and a physiological effect ensues. Indirect proof lies in the caveolae structure and the activity of H^+-ATPase hydrolase, and so on, when cells were dissolved by eradicator with non-hydronium and observed by electron microscopy. Many analyses of the caveolae are in progress both here and abroad (Qiu 1999; Zhao *et al.* 2002; Wen *et al.* 2004).

ACKNOWLEDGEMENTS

We are indebted to Prof. Weidong Zhou of the Testing Centre of Yangzhou University for excellent technical assistance. This work was supported by the Testing Centre of Yangzhou University.

REFERENCES

Anderson RGW (1991) Molecular motors that shape endocytic membrane. In: Steer CJ, Hanover JA (Eds) *Intracellular Trafficking of Proteins*, Cambridge University Press, Cambridge, pp 13-46

Caldwell RB, Slapnick SM (1992) Freeze fracture and lanthanum studies of retinal microvasculature in diabetic rats. *Investigative Ophthalmology and Visual Science* **33**, 1610-1619

Ding B, Itaya A, Woo YM (1999) Plasmodesmata and cell-to-cell communication in plants. *International Review of Cytology* **190**, 251-315

Fujimoto T, Nakade S, Miyawaki A, Mikoshiba K, Ogawa K (1992) Localization of inositol 1,4,5-trisphosphate receptor-like protein in plasmal caveolae. *The Journal of Cell Biology* **119**, 1507-1513

Lisantl MP, Tang Z, Scherer PE, Kubler E, Koleske AJ, Sargiacomo M (1995) Caveolae, transmembrane signalling and cellular transformation. *Molecular Membrane Biology* **12**, 121-124

Ouyang XZ, Xie SP (1996) Plasmalemma invaginations in cultured callus of *Stevia rebaudiana*: ultrastructure and ultracytochemical localization. *Acta Botanica Sinica* **38**, 589-593

Qiu QS (1999) Structure and function of plant plasma membrane H^+-ATPase. *Chinese Bulletin of Botany* **16**, 122-126

Guo R, Zhang HJ, Dong DX, Cao YY, Dai CB, Qin HX, He J, Cai HR, Shi NY, Chu JY, Zhou J, Zhou G, Chen SF, Gong CM, Guo YJ, Wang LY, Tang EH (1990) *Molecular Cell Biology*, Beijing Medical University and Consonancy Medical University of China Combined Press, pp 121-122

Robards AW, Lucas WJ (1990) Plasmodesmata. *Annual Review of Plant Physiology* **26**, 13-29

Smart EJ, Ying YS, Anderson RG, (1995) Hormonal regulation of caveolae internalization. *The Journal of Cell Biology* **131**, 929-938

Travis (1993) Cell biologists explore "tiny caves". *Science* **262**, 1206-1209

Wen B, Bin JH, Pan RC, Wang X (2004) Isolation of mung bean plasma membrane vesicles and the analysis of hydrolysis activity of PM H^+-ATPase. *Chinese Bulletin of Botany* **21**, 319-325

Willingham MC, Pastan L (1984) *International Review of Cytology* **92**, 51-92

Yamada E (1955) The fine structure of the gall bladder epithelium of the mouse. *Biophysics and Biochemistry of Cytology* **1**, 445-458

Yu SW, Tang ZC, Shen YG, Hong MM, Xia ZA, Song HY, Zhao YJ, Wei ZM, Zhu ZP, Wang HZ, Zhou ZK, Cao ZG (1998) Plant Physiology and Molecular Biology. *Science Press*, pp 135-153

Zhao FG, Shu HR (2002) Relationship between Na^+-H^+ antiport and polyamines in plasma membrane vesicles prepared from barley roots under salt treatment. *Journal of Plant Physiology and Molecular Biology* **28**, 333-338