

An electron microscopic-cytochemical localization of plasma membrane Ca²⁺-ATPase activity in poplar apical bud cells during the induction of dormancy by short-day photoperiods

JIAN LING CHENG^{1,2}, JI HONG LI^{1,3}, PAUL H LI^{1,*}, TONY HH CHEN⁴

1 Laboratory of Plant Hardiness, Department of Horticultural Science and Plant Biological Sciences Program, University of Minnesota, St. Paul, MN 55108, USA

2 Visiting professor from Institute of Botany, Chinese Academy of Sciences, Beijing, China.

3 Visiting scientist from Beijing Agricultural University, Beijing, China.

4 Department of Horticulture and Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR 97331, USA

ABSTRACT

Plasma membrane (PM) Ca²⁺-ATPase activity in poplar apical bud meristematic cells during short-day (SD)-induced dormancy development was examined by a cerium precipitation EM-cytochemical method. Ca²⁺-ATPase activity, indicated by the status of cerium phosphate precipitated grains, was localized mainly on the interior face (cytoplasmic side) of the PM when plants were grown under long days and reached a deep dormancy. A few reaction products were also observed on the nuclear envelope.

When plant buds were developing dormancy after 28 to 42 d of SD exposure, almost no reaction products were present on the interior face of the PM. In contrast, a large number of cerium phosphate precipitated grains were distributed on the exterior face of the PM. After 70 d of SD exposure, when buds had developed a deep dormancy, the reaction products of Ca²⁺-

* Corresponding author.

Tel: 1612 624 1757; Fax: +1612 624 4941; E-mail: lixxx008@tc.umn.edu

Poplar Ca²⁺-ATPase activity during dormancy

ATPase activity again appeared on the interior face of the PM. The results seemed suggesting that two kinds of Ca²⁺-ATPases may be present on the PM during the SD-induced dormancy in poplar. One is the Ca²⁺-pumping ATPase, which is located on the interior face of the PM, for maintaining and restoring the Ca²⁺ homeostasis. The other might be an ecto-Ca²⁺-ATPase, which is located on the exterior face of the PM, for the exocytosis of cell wall materials as suggested by the fact of the cell wall thickening during the dormancy development in poplar.

Key words : *Ca²⁺-homeostasis, Ca²⁺-ATPase, ecto-Ca²⁺-ATPase, poplar dormancy, populus deltoides.*

INTRODUCTION

Cytoplasmic calcium concentration plays an important role in various physiological processes in plants by acting as a “second messenger” in the transduction of endogenous or exogenous signals[1-3]. Endogenous and exogenous hormones and environmental stimuli, such as temperature, light and wind, can increase cytosolic Ca²⁺ levels[1-3]. A low temperature-induced transient increase in cytosolic Ca²⁺ concentration is necessary for the expression of cold-acclimation-specific genes and the development of freezing tolerance in alfalfa[4],[5].

A prolonged high level of Ca²⁺ in the cytosol is harmful, and often results in metabolic dysfunction and structural damage, when such as chilling-sensitive plants are subjected to chilling stress[6],[7]. Plasma membrane (PM) Ca²⁺-ATPase plays a crucial role in controlling cytosolic Ca²⁺ concentration[8-14]. The PM calcium-pumping, ATPase uses the energy supplied by the hydrolysis of ATP to transport calcium ions from the cytoplasm to the extracellular space. Because of its high affinity for calcium, this enzyme can either reduce the cytoplasmic concentration of free calcium ions to extremely low levels (thus maintaining a Ca²⁺ homeostasis), or restore it after a specific stimulus causes an increase in cytosolic Ca²⁺ concentration[11],[15].

We previously found a dynamic change in calcium distribution in poplar apical bud meristematic cells when dormancy was induced by short days (SD)[16]. For example, during SD exposures of up to 20 d (prior to a measurable level of dormancy), Ca²⁺ increased in cytosol and nuclei. Whilst from day 28 to 49 of SD exposure, when dormancy was developing rapidly, it increased further in the same compartment. After 70 d of SD exposure, when deep dormancy had been reached, the levels of Ca²⁺ in cytosol and nuclei decreased. This suggests that Ca²⁺ dynamics are tightly regulated during the development of dormancy in poplar buds.

In this study, we used an electron-microscopic cytochemical method to examine the change in PM Ca^{2+} -ATPase activity in poplar apical bud meristematic cells during SD-induced dormancy.

MATERIALS AND METHODS

Plant materials

Poplar (*Populus deltoides* Bartr. ex March) plants were established as previously described [16]. They were grown in a greenhouse with 16 h light (long day, LD) at 25/21 °C day/night (D/N). When plants were 40-50 cm tall, they were transferred to a growth chamber with 8 h light (short days, SD) at 25/21 °C D/N. The SD experiment was designed so that all samples for fixation could be collected at the same time to avoid any environmentally caused discrepancy during sample preparation.

Measurement of dormancy

After 10, 20, 28, 42 or 70 d of SD exposure, one set of 6-8 plants at each date was transferred from the SD chamber to a LD greenhouse at 25/21 °C D/N. Plants were manually defoliated and the shoot tips, about 2.5 cm long, were removed. The degree of dormancy was expressed as the number of days to first bud break (i.e., the first visible shoot growth) in the defoliated and decapitated plants.

EM-cytochemical localization of Ca^{2+} -ATPase activity

Sample collection

Apical buds were collected from plants: a) grown under LD; b) exposed to SD for 28, 42, or 70 d; or c) exposed to SD for 42 d, when plants had developed some level of dormancy, and then transferred to LD for 30 d to break dormancy.

Ca^{2+} -ATPase EM-cytochemical procedures

Ca^{2+} -ATPase activity was detected according to the one step lead method of Ando et al.[17], with minor modifications[18],[19]. Cerium (CeCl_3) was used instead of lead, and lower concentration of calcium was used in the reaction medium for the activation of Ca^{2+} -ATPase. After the bud scales and young leaves were removed, the buds were cut into $0.5 \times 0.5 \times 0.5$ mm slices, and immediately fixed in 4 % paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 h at room temperature. After fixation, the samples were washed twice in cacodylate buffer (pH 7.2), then twice with Tris-malate buffer (pH 7.5), for 25 min each at 4 °C. The samples were then incubated in a reaction medium consisting of 50 m M Tris-malate buffer (pH 7.5), 2 m M ATP-Na, 1.5 m M CaCl_2 and 3 m M CeCl_3 for 1 h at 23 °C.

Four control reactions were performed to demonstrate the specificity of the reaction products: 1) ATP was omitted from the reaction medium.

2) The samples were pre-treated with 5 m M EGTA in Tris-malate buffer (pH 7.5) for 30 min, and then incubated in a medium without CaCl_2 , to which 5 m M EGTA was added.

3) The samples were pre-treated for 30 min at room temperature in a Tris-malate buffer (pH 7.5) containing 10 m M sodium ortho-vanadate (Na_3VO_4), an inhibitor of PM Ca^{2+} -ATPase activity[20], and then incubated in the complete reaction medium containing 10 m M Na_3VO_4 .

4) The samples were pre-treated for 30 min at room temperature in Tris-malate buffer (pH 7.5) containing 0.1 m M Erythrosin B, which is a specific inhibitor of Ca^{2+} -ATPase activity[8],[21], and then incubated in the complete reaction solution containing 0.1 m M Erythrosin B.

After incubation, all samples were post-fixed in 4 % glutaraldehyde in cacodylate buffer (pH 7.2) for 3 h at 4 °C. They were then fixed in 1 % osmium tetroxide (OsO_4) in cacodylate buffer (pH

Poplar Ca²⁺-ATPase activity during dormancy

7.2) overnight at 4 °C. Thereafter, the samples were dehydrated in an ethanol series, and embedded in EMbed 812 (EMS, Fort Washington, PA, USA). The embedded samples were then sectioned with a diamond knife and an RMC (Tucson, AZ, USA) MT 7000 ultramicrotome. The sections were stained with uranyl acetate, then observed and photographed under a Philips (F.E.I. Company, Tacoma, WA, USA) CM12 TEM operated at 60 kV. At the same time, one group of samples was fixed according to routine glutaraldehyde-osmium tetroxide procedures. These samples were also used as the control for Ca²⁺-ATPase cytochemical localization.

RESULTS

Dormancy development

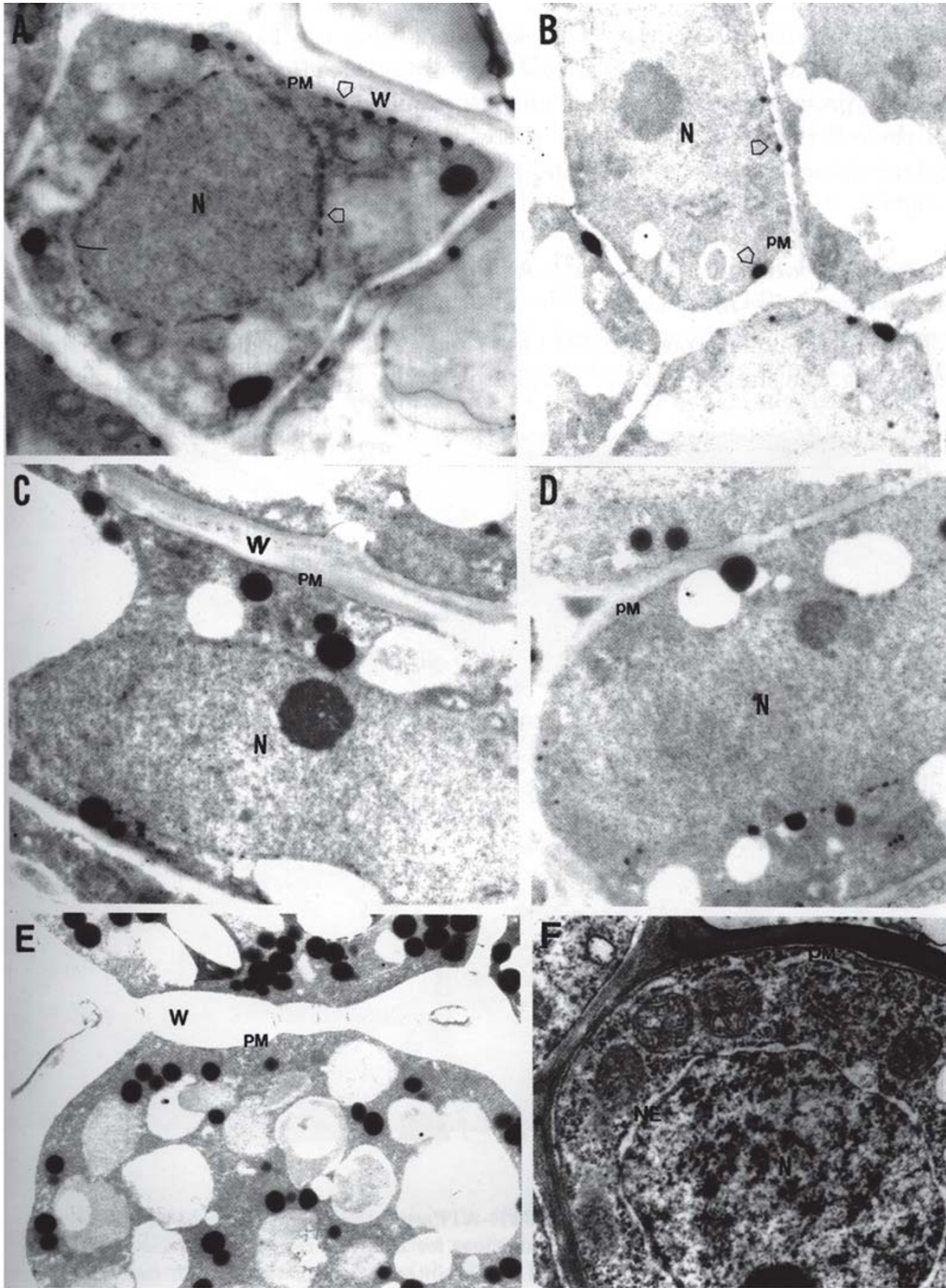
The growth rate of the poplar plants decreased immediately upon transferring from LD to a SD/warm temperature regime; growth stopped completely after 20 d of SD exposure (data not shown). At this point, however, there was no significant change in dormancy status. The number of days to bud break was 14 and 30 for plants exposed to SD for 28 and 42 d, respectively. After 70 d of SD exposure, it took 90 d of LD exposure before any bud regrowth was detected.

Localization of Ca²⁺-ATPase activity in apical bud meristematic cells of poplar grown in LD

When samples were incubated in the complete reaction medium containing ATP, CaCl₂ and CeCl₃, Ca²⁺-ATPase hydrolyzed the substrate ATP to ADP and inorganic phosphate, which was precipitated by cerium ions (i.e., $\text{ATP} + \text{Ce}^{+3} \rightarrow \text{ADP} + \text{CePO}_4 \downarrow$). The reaction product cerium phosphate formed a fine-grained deposit that was sufficiently electron-dense to be detected with electron microscopy. In EM observations of the section samples, these cerium phosphate-precipitated grains were localized mainly on the cytoplasmic side of the PM, indicating where the Ca²⁺-ATPase activity reaction had occurred (Fig 1A, B, at arrows). A smaller number of reaction products were also observed on the nuclear envelope (NE; Fig 1A, at arrow). The control reactions included samples in which: 1) ATP was omitted (result not shown); 2) CaCl₂ was omitted and 5 m M EGTA was added (Fig 1C); 3) 10 m M Na₃VO₄ was added (Fig 1D, E); or 4) 0.1 m M Erythrosin B was added (result

▷ Fig 1.

EM-cytochemical localization of Ca²⁺-ATPase activity in poplar apical bud cells. A, B: Samples were collected from plants grown under LD. The reaction products, cerium phosphate precipitated grains, are an indication of Ca²⁺-ATPase activity sites. These grains were mainly localized on the interior face of the plasma membrane (PM). A smaller number of cerium phosphate precipitates was also observed on the nuclear envelope (NE; at arrows). A, 14000 × ; B, 10000 × . C-F: Controls for demonstrating the truthfulness of reaction products. C, Cells of LD-grown plants. CaCl₂ was omitted and 5 m M EGTA was added to the reaction solution. D and E: Cells from either LD-grown plants, or those with 42 d of SD exposure. Ten m M Na₃VO₄ was added to the reaction solution. F, Cells were fixed following routine glutaraldehyde-osmium tetroxide procedures. No visible cerium phosphate precipitated grains were observed on the PM or NE in any of these control samples. C, 12000 × ; D, 12000 × ; E, 10000 × ; F, 25000 × . W: Cell wall. N: Nucleus.



Poplar Ca^{2+} -ATPase activity during dormancy

not shown). In all four controls, either no or few visible electron-dense precipitate grains were observed on the PM and the NE. This indicates that the Ca^{2+} -ATPase detection method was capable of specifically identifying the location of Ca^{2+} -ATPase activities. In addition, the cells fixed according to routine glutaraldehyde-osmium tetroxide procedures did not show any electron-dense precipitate grains on the PM and the NE (Fig 1F). Our results, therefore, demonstrate that the location of the electron-dense grains of cerium phosphate is a reliable indicator of Ca^{2+} -ATPase activity.

Changes in localization of Ca^{2+} -ATPase activity in poplar apical bud cells during dormancy induction

For poplar plants exposed to SD for 28 and 42 d, when bud dormancy was rapidly developing, the sites of PM Ca^{2+} -ATPase activity were clearly altered (Fig 2A-C;3A-D). No or only few cerium phosphate precipitate grains were present on the interior face of the

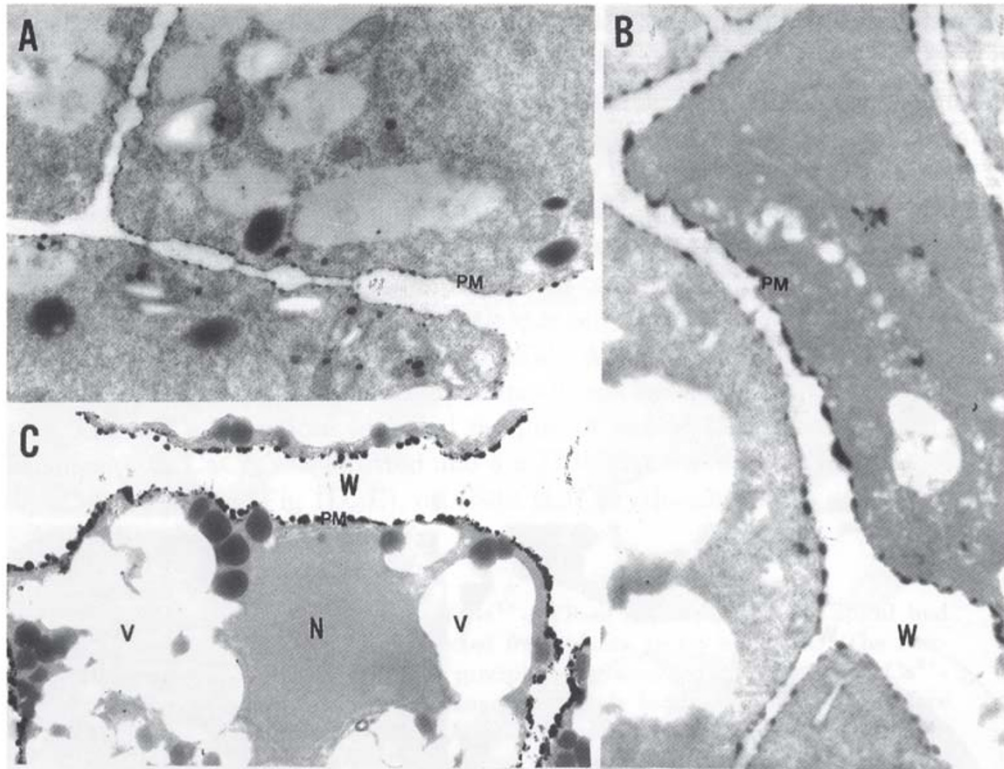


Fig 2.

EM-cytochemical localization of Ca^{2+} -ATPase activity in the apical bud cells of poplar plants grown under SD conditions for 28 d. No reaction products of the enzymatic activity were observed on the interior face of the PM. In contrast, many cerium phosphate precipitated grains were seen on the exterior face of the PM. V: Vacuole. A, 12000 \times ; B, 19000 \times ; C, 12000 \times .

PM. In contrast, after 28 d of SD exposure, abundant cerium phosphate precipitate grains were seen on the exterior face of the PM (Fig 2A-C), showing an ecto- Ca^{2+} -ATPase characteristic[22-24]. After 42 d of SD exposure, when a higher degree of dormancy had developed, both the size and the number of the cerium precipitate grains on the exterior face of the PM had decreased (Fig 3A-D). After 70 d of SD exposure, when buds were in deep dormancy, the reaction products of Ca^{2+} -ATPase activity reappeared on the interior face of the PM (Fig 4A, B). Cerium precipitates were also localized on the NE (Fig 4A).

For plants exposed to SD for 42 d and then grown under LD, it took about 30 d until bud break. Immediately after growth resumed, Ca^{2+} -ATPase activity was localized mainly on the interior face of the PM. This was similar to buds that were observed from plants grown under LD (Fig 4C and D, at arrows), except that a few cerium precipitate grains were also observed on the NE (Fig 4D, at arrow).

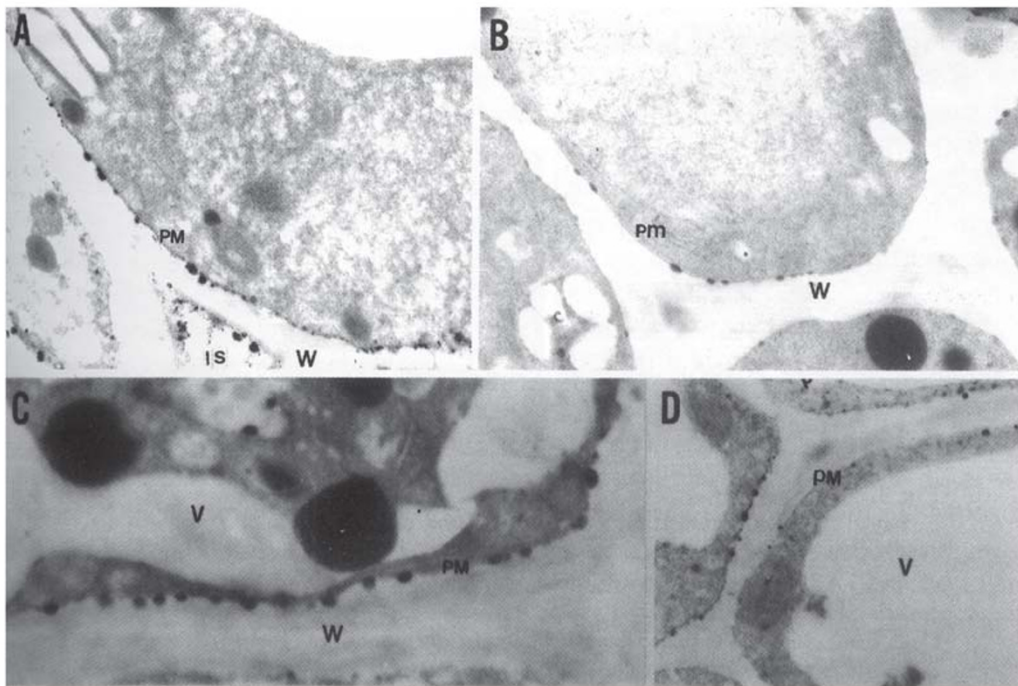


Fig 3.

Localization of Ca^{2+} -ATPase activity in apical bud cells of poplar plants after 42 d of SD exposure. The reaction products of enzymatic activity were still distributed on the exterior face of the PM. The number and the size of cerium phosphate precipitated grains, however, appeared to be less and smaller than those after 28 d of SD exposure.

A, B, D, 15000 \times ; C, 19000 \times .

Poplar Ca^{2+} -ATPase activity during dormancy

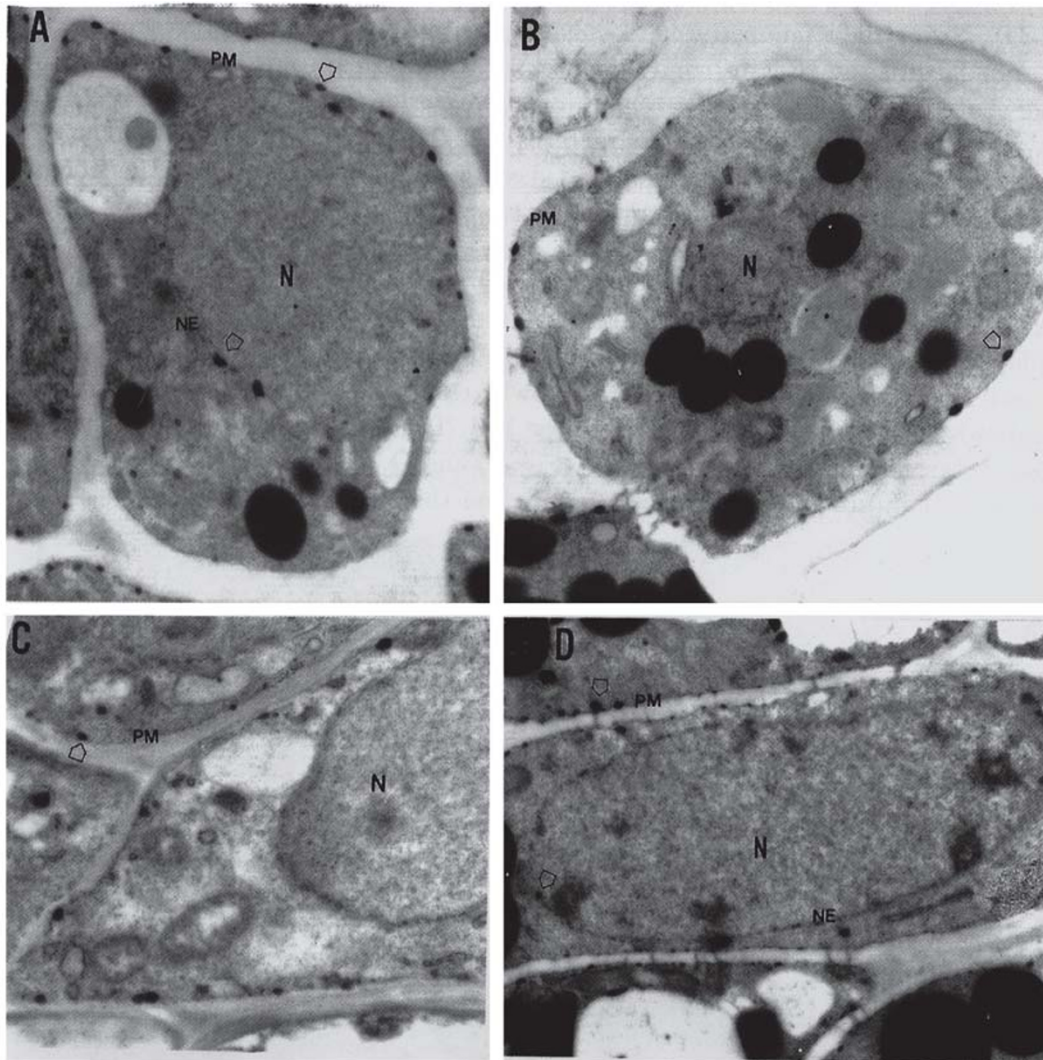


Fig 4.

EM-cytochemical localization of Ca^{2+} -ATPase activity in poplar apical bud cells. A and B: Cells from plants exposed to SD for 70 d. The reaction products of Ca^{2+} -ATPase activity reappeared on the interior face of the PM. A few cerium phosphate precipitated grains were also appeared on the NE. C and D: Cells from 42-d/SD exposed plants when the dormancy was just broken after a 30-d/LD exposure. The sites of the Ca^{2+} -ATPase activity were similar as those before the dormancy was developed. The reaction products were distributed on the interior face of the PM and a few cerium phosphate precipitated grains (at arrow) were also found on the NE (at arrow). A, B, 16000 \times ; C, D, 13000 \times .

DISCUSSION

The PM Ca^{2+} -ATPase has been studied extensively in both animal and plant cells using either biochemical or cytochemical methods[8-10],[13],[14], [18],[25],[26]. In biochemi-

cal assay with cell free preparation, Mg^{2+} ions must be added to the reaction medium in addition to the Ca^{2+} for the activation of the Ca^{2+} -ATPase[8],[9],[13]. However, for cytochemical assay with integral cells, the Ca^{2+} -ATPase activity can proceed in the absence of added Mg^{2+} in the reaction medium, because endogenous Mg^{2+} ions already exist in the cells[18],[23],[26]. We tested the poplar Ca^{2+} -ATPase activity in the reaction media with and without added Mg^{2+} ions. Our results agreed with the literature that no added Mg^{2+} is needed in the reaction medium for the cytochemical assay of poplar apical buds (data not shown). Also, in this study, we used a 4 % paraformaldehyde for the fixation instead of the classic glutaraldehyde fixative, because PM Ca^{2+} -ATPase activity could be partially inhibited by the latter[18],[27]. Our results (Figs 1-4) with the former fixative yielded a much better resolution than the latter (data not shown).

The one-step lead detection method of Ando et al.[17] has been used by many scientists[18],[24-26],[28-32] for the cytochemical localization of Ca^{2+} ATPase activity. The reliability and specificity of this method has also been demonstrated by locating the bands of Ca^{2+} -ATPase activity on polyacrylamide gel film, using the same conditions as for EM-cytochemical localization[33]. EM-cytochemical localization experiments have revealed that the reaction products of the PM Ca^{2+} -pump ATPase activity were localized on the interior face of the PM[18],[20],[22], [26],[34],[35]. Our findings agreed with the literature, but only when poplar plants were actively growing under LD or reached a full dormancy, e.g., after 70 d of SD exposure. Between the 28 and 42 d of SD exposure, when the dormancy was developing, no reaction products of the enzymatic activity were seen on the interior face of the PM. The dynamic of Ca^{2+} -ATPase activity appears to be an adaptive nature corresponding to the alterations of the intracellular Ca^{2+} concentration [16].

This enzyme has high affinity for calcium, and can reduce internal calcium concentration to an extremely low level[11],[14],[15], [34]. Active calcium pumping is necessary in order to maintain a Ca^{2+} -homeostasis, as plants such as poplar are actively growing. When poplar plants were exposed to SD, e.g., between the 28-42 d period, the dormancy was developing, and intracellular Ca^{2+} concentration was increased[16]. Naturally, it may be expected that during this period of SD exposure the PM Ca^{2+} pumping should be very low or inactive, and thus would result in no cerium phosphate grains on the interior face of the PM. When the plant has completed its dormancy process, we believe the plant should be back to the status of a resting low Ca^{2+} concentration, as evidenced by the observation in poplar after a 70-d SD exposure in which the PM Ca^{2+} pumping was restored (Fig 4) and a low concentration of intracellular Ca^{2+} was observed[16].

As shown in Fig 2 and 3, many reaction products were present on the exterior face of the PM during the dormancy development. We did not attempt to identify them, but from the literature, it seems suggesting that the cerium precipitated grains on the exterior face of the PM may be the reaction products of the ecto- Ca^{2+} -ATPase activity[23],[24]. An ecto- Ca^{2+} -ATPase has been reported in animal cells and its reaction products were located on the exterior face of the PM[22-24],[36-40]. Ca^{2+} -ATPase and ecto- Ca^{2+} -

Poplar Ca²⁺-ATPase activity during dormancy

ATPase may be distinguished by using different concentrations of CaCl₂ in the cytochemical reaction medium. The activity of former could be obtained at a range of 0.5 ~ 1.0 mM CaCl₂[18],[20], [22],[23], whereas the latter needed a range of 5.0 ~ 10.0 mM CaCl₂[22-24], [40]. In our study, we used 1.5 mM CaCl₂ in the reaction medium. It appeared that a concentration of 1.5 mM CaCl₂ may be workable for both enzymes in the poplar. Ecto- Ca²⁺-ATPase has been known in animal cells for many years, its biological function has been considered to be involved in many aspects of physiological and biochemical processes in various organs and tissues[40]. Thirion et al.[24] suggested that ecto- Ca²⁺-ATPase activity may associate with the exocytotic secretion of neuropeptide granules containing ATP. We have observed that when poplar dormancy was rapidly developing, the cell wall was markedly thickened[16]. Therefore, we speculate that the ecto- Ca²⁺-ATPase in poplar may play some role in the exocytosis of cell wall materials. Its activity may involve in the kinetics of the fusion of transporting vesicles with plasma membrane, and of the secretion of the materials in the vesicles into cell wall.

REFERENCES

- [1] Hepler PK, Wayne RO. Calcium and plant development. *Ann Rev Plant Physiol* 1985; **36**:397-439.
- [2] Poovaiah BW and Reddy ASN. Calcium and signal transduction in plants. *Critical Rev in Plant Sci* 1993; **12**:185-211.
- [3] Bush DS. Calcium regulation in plant cells and its role in signaling. *Annu. Rev. Plant Physiol. Plant Mol Biol* 1995; **46**:95-122.
- [4] Monroy AF, Dhindsa RS. Low-temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25. *Plant Cell* 1995; **7**:321-31.
- [5] Monroy AF Sarhan F, Dhindsa RS. Cold-induced changes in freezing tolerance, protein phosphorylation, and gene expression: evidence for a role of calcium. *Plant Physiol* 1993; **102**:1227-35.
- [6] Minorsky PV. A heuristic hypothesis of chilling injury in plants: a role for calcium as the primary physiological transducer of injury. *Plant, Cell and Environment* 1985; **8**:75-94.
- [7] Wang H, Jian LC. Changes of level of Ca²⁺ in the cells of rice seedlings under low temperature stress. *Acta Bot Sinica* 1994; **36**:587-97.
- [8] Rasi-Caldogno F, Pugliarello MC, Olivari C and de Michelis MI. Identification and characterization of the Ca²⁺-ATPase which drives active transport of Ca²⁺ at the plasma membrane of radish seedlings. *Plant Physiol* 1989; **90**:1429-34.
- [9] Rasi-Caldogno F, Carnelli A, de Michelis MI. Plasma membrane Ca-ATPase of radish seedlings. II. Regulation by calmodulin. *Plant Physiol* 1992; **98**:1202-6.
- [10] Carafoli E. Calcium pump of the plasma membrane. *Physio Rev* 1991; **71**:129-53.
- [11] Evans DE, Briars SA, Williams LE. Active calcium transports by plant cell membranes. *J Experimental Bot* 1991; **42**:285-303.
- [12] Thomson LJ, Xing T, Hall JL, Williams LE. Investigation of the calcium transporting ATPase at the endoplasmic reticulum and plasma membrane of red beet (*Beta vulgaris*). *Plant Physiol* 1993; **102**: 553-64.
- [13] Bonza C, Carnelli A, de Michelis MI, Rasi-Caldogno F. Purification of the plasma membrane Ca²⁺-ATPase from radish seedlings by calmodulin agarose affinity chromatography. *Plant Physiol.* 1998; **116**:845-51.
- [14] Evans DE, Williams LE. P-type Ca²⁺-ATPase in higher plants-biochemical, and functional properties. *Biochim et Biophys Acta* 1998; **1376**:1-25.

- [15] Rega AF, Garrahan PJ. eds. The Ca²⁺ pump of plasma membranes. CRC Press, Boca Raton, FL. 1986.
- [16] Jian LC, Li PH, Sun LW, Chen THH. Alterations in ultrastructure and subcellular localization of Ca²⁺ in poplar apical bud cells during the induction of dormancy. *J Experimental Bot* 1997; **48**:1195-207.
- [17] Ando T, Fujimoto K, Mayahura H, Miyajima H, Ogawa K. A new one-step method for the histochemistry and cytochemistry of Ca²⁺-ATPase activity. *Acta Histochem. et Cytochem* 1981; **14**:705-15.
- [18] Kortje KH, Freihofer D, Rahmann H. Cytochemical localization of high-affinity Ca²⁺-ATPase activity in synaptic terminals. *J Histochem Cytochem* 1990; **38**:895-900.
- [19] Jian LC, Li JH, Li PH, Chen THH. Cytochemical localization of calcium and Ca²⁺-ATPase activity in plant cells under chilling stress: a comparative study between the chilling-sensitive maize and chilling insensitive winter wheat. *Plant Cell Physiol.* 1999; **40**:1061-71.
- [20] Gioglio L, Rapuzzi G and Quacci D. Ca²⁺-ATPase and Na⁺, K⁺-ATPase activities in the fungi form papilla of the tongue of *Rana esculenta*. *J Morphology.* 1991; **210**:117-25.
- [21] Williams LE, Schueler SB, Briskin DP. Further characterization of the red beet plasma membrane Ca²⁺-ATPase using GTP as an alternative substrate. *Plant Physiol* 1990; **92**:747-54.
- [22] Barry MJ. Ecto-calcium-dependent ATPase activity of mammalian taste bud cells. *J Histochem and Cytochem* 1992; **40**:1919-28.
- [23] Maxwell WL, McCreath BJ, Graham DI, Gennarelli TA. Cytochemical evidence for redistribution of membrane pump Ca²⁺-ATPase and ecto- Ca²⁺-ATPase activity, and Ca²⁺ influx in myelinated nerve fibres of the optic nerve after stretch injury. *J Neurocytol* 1995; **24**:925-42.
- [24] Thirion S, Troadec JD, Nicaise G. Cytochemical localization of ecto-ATPase in rat neurohypophysis. *J Histochem Cytochem* 1996; **44**:103-11.
- [25] Davis WL, Jones RG, Goodman DBP. Electron microscopic cytochemical localization of Ca²⁺-ATPase in toad urinary bladder. *J Histochem cytochem* 1987; **35**:39-48.
- [26] Pappas GD, Kriho V. Fine structural localization of Ca²⁺-ATPase activity at frog neuromuscular junction. *J Neurocytol* 1988; **17**:417-23.
- [27] Ogawa KS, Fujimoto K, Ogawa K. Ultracytochemical studies of adenosine nucleotidases in aortic endothelial and smooth muscle cells - Ca²⁺-ATPase and Na⁺, K⁺-ATPase. *Acta Histochem Cytochem* 1986; **19**:601-10.
- [28] Belitser NV, Zaalishvili GV, Sytnianskaja NP. Ca²⁺-binding sites and Ca²⁺-ATPase activity in barley root tip cells. *Protoplasma* 1982; **111**:63-78.
- [29] Eleftheriou EP, Hall JL. The extrafloral nectars of cotton. II. Cytochemical localization of ATPase activity and Ca²⁺ binding sites, and selective osmium impregnation. *J Experimental Bot* 1983; **34**:1066-79.
- [30] Mata M, Fink DJ. Ca²⁺-ATPase in the central nervous system: an EM cytochemical study. *J Histochem Cytochem* 1989; **37**:971-80.
- [31] Maggio K, Watrin A, Keicher E, Nicaise G, Hernandez-Nicaise ML. Ca²⁺-ATPase and Mg²⁺-ATPase in aplasia glial and interstitial cells: an EM cytochemical study. *J Histochem Cytochem* 1991; **39**:1645-58.
- [32] Eisenmann DR, Salama AH, Zaki AME. Effects of vinblastine on calcium distribution pattern and Ca²⁺, Mg²⁺-ATPase in rat incisor maturation ameloblasts. *J Histochem Cytochem* 1992; **40**:143-51.
- [33] El-Sherif G, van Noorden CJF, Bacsy E. Specificity of the metal-salt method for the localization of Ca²⁺-ATPase activity studied on rat adenohypophysis tissue in a model system polyacrylamide gel films. *Histochem J* 1990; **22**:51-62.
- [34] Penniston JT. Plasma membrane Ca²⁺-ATPase as active Ca²⁺ pumps. In Cheung W Y. ed. Calcium and Cell Function, Academic Press, New York. 1983; vol. IV:99-110.
- [35] Mughal S, Cuschieri A, Al-Bader AA. Intracellular distribution of Ca²⁺-Mg²⁺-ATPase in various tissues. *J Anatomy* 1989; **162**:111-25.
- [36] Hamlyn JM, Senior AE. Evidence that Ca²⁺- or Mg²⁺-activated ATPase in rat pancreas is a plasma membrane ecto-enzyme. *Biochem J* 1983; **214**:59-65.

Poplar Ca²⁺-ATPase activity during dormancy

- [37] Nagy AK, Shuster TA and Delgado-Escueta AV. Ecto-ATPase of mammalia synaptosomes: identification and enzyme characterization. *J Neurochem* 1986; **4**:976-87.
- [38] Lin SH, Russell WE. Two Ca²⁺-dependent ATPase in rat liver plasma membrane. *J Biol Chem* 1988; **263**:12253-62.
- [39] Cheung PH, Dowd EJ, Porter JE, Li LS. A Ca²⁺-ATPase from rat parotid gland plasma membranes has the characteristics of an ecto-ATPase. *Cell Signal* 1992; **4**:25-36.
- [40] Plesner L. Ecto-ATPase: Identities and functions. *Int Rev Cytology* 1995; **158**:141-56.

Received April-17-2000. Revised May-10-2000. Accepted May-15-2000.