Plant Physiol. (1988) 86, 899–903
0032-0889/88/86/0899/05/$01.00/0

Early Activation by Ethylene of the Tonoplast H^+-Pumping ATPase in the Latex from *Hevea brasiliensis*

Received for publication February 17, 1987 and in revised form September 24, 1987

XAVIER GIDROL*[1], HERVÉ CHRESTIN, GILLES MOUNOURY, AND JEAN D'AUZAC
Laboratoire de Physiologie Végétale, Orstom, BP V.51 Abidjan, Cote D'Ivoire (X.G., H.C., G.M.); *and Laboratoire de Physiologie Végétale appliquée USTL de Montpellier 34088 Montpellier Cedex, France* J.D'A.)

ABSTRACT

The treatment of *Hevea brasiliensis* (rubber tree) bark by chloro-2-ethyl phosphonic acid (ethrel), an ethylene-producing compound, induces a significant increase in the tonoplast H^+-translocating ATPase activity in the latex during the first 24 hours after the application of the stimulating agent. Moreover, the tonoplast-bound ATPase is highly activated when vacuoles (lutoids) are resuspended in ultrafiltrated cytosol. This effect is amplified during ethrel stimulation. Preliminary assays to characterize the endogenous effector(s) suggest that the activator(s) could be a heat-resistant compound with a low molecular weight, most likely an anion. The activation of the tonoplast-bound ATPase and the associated activation of the protons translocation across the lutoid membrane, could explain the cytosolic alkalinization observed in latex following the ethrel treatment of *Hevea* bark, which results in an enhanced rubber production.

Hevea latex is a specialized fluid cytoplasm which is expelled from wounded syncytial laticiferous system. Besides rubber particles, the latex contains various organelles including lutoids (15% of total volume of latex). The lutoids are single membrane microvacuoles with lysosomal characteristics (27) which can be easily isolated and purified by simple centrifugation. The occurrence of a Mg^{2+}-dependent ATPase bound to the tonoplast has been demonstrated in lutoids from *Hevea* latex (10). Its properties have been extensively described in intact vacuoles (8, 14), in reconstituted tonoplast vesicles, and with the purified enzyme (22–25). This tonoplast-bound enzyme functions as an electrogenic pump, translocating protons from cytosol into vacuoles (8, 22). Moreover, the lutoid tonoplast possesses a redox system which uses NADH as the electron donor and Cyt *c* as the exogenous acceptor. This membrane electron carrier system is able to release protons from the lutoids into the cytosol (9). The functioning of these two opposing proton pumps, as determined under physiological conditions, intact lutoids incubated in ultrafiltrated cytosol, shows that the two pumps operate as a biophysical pH-stat that is capable of regulating the cytosolic pH in the physiological pH range towards an efficient metabolism (5).

Ethrel,[2] an ethylene-producing compound, is commonly used on *Hevea brasiliensis* to stimulate the production of latex (11). Treatment of the bark with this hormone induces, in latex, a cytosolic alkalinization coupled with an acidification of the vacuolar compartment (lutoids) (3). The cytosolic alkalinization enhances the catabolism of sugars and latex regeneration in laticiferous cells by activating several pH-dependent enzymes (16, 17). This metabolic stimulation induces a large, but transient, increase of latex production. Thus, cytosolic alkalinization is so strongly associated with a high latex production that it could be regarded as a selective criterion in the research of high yield clones (13).

Ethylene is involved in many aspects of plant growth and development (19), and induces numerous physiological effects in plant tissues, such as increases in the rate of respiration (28), increases in activities of enzymes, such as chitinase (2), phenylalanine ammonia-lyase (4), and Cyt *c* oxidase (1), and changes in the level of mRNAs (29). Nevertheless, the fine molecular mechanisms whereby ethylene induces its physiological effects and the level of control exerted upon these mechanisms remain unknown.

To understand the mechanism of action of ethylene on the metabolism of laticiferous cells, we have tried to determine whether cytosolic alkalinization, after the ethrel treatment could be correlated with an activation of the tonoplast ATPase activity. The mechanism whereby such an activation of the tonoplast-bound H^+-ATPase could be induced by ethrel treatment is studied and discussed.

MATERIALS AND METHODS

Plant Material. Twelve *Hevea* trees, GT 1 clone, were selected for the homogeneous biochemical properties of their latex. After some preliminary controls (lutoid ATPase activity, pH of latex, pH of lutoids) the trees were left to recover, without any treatment, for 1 week. Hence, the latex could attain a 'stationary state' unaffected by the regeneration process which follows frequent tappings, generally twice a week.

Ethrel Treatment. The trees were divided into six homogeneous groups, one control and five ethrel-treated groups. Ethrel (200 mg active material/tree) was diluted at 5% (v/v) with palm oil. This emulsion was applicated with a brush on a 25 mm wide band of virgin bark just below the cut, which was previously scratched in order to eliminate most of the suberized part of the bark. The control tree was treated in the same way with palm oil only. Ethrel was applied 72, 48, 36, 24, and 12 h before the first cut. All the rubber trees (both control and treated groups) were tapped on the same day, so that any artefact due to intercut variations was avoided.

Preparation of Lutoids Fraction. The fresh latex was collected in glass vessels held in melting ice. The first 20 ml were discarded in order to avoid possible bacterial contamination and damaged particle content. The fresh latex was then immediately centrifuged at 35,000g for 20 min at 4°C. The supernatant serum (cy-

[1] Present address: Laboratoire de Physiologie Végétale, I.N.R.A., C.R. de Bordeaux, B.P. 131, 33140 Pont de la Maye.

[2] Abbreviations: ethrel, chloro-2-ethyl phosphonic acid; DOC, sodium deoxycholate; NEM, *N*-ethylmaleimide.

toplasm) and the polyisoprenoid particle fractions were discarded. The pellet resuspended in 5 volumes of a 50 mM Hepes-Mes-Tris (pH 7), 300 mM mannitol buffer, formed the crude lutoid fraction. The crude lutoid fraction was washed three times with the same buffer. The sediment obtained by centrifugation of the lutoidic suspension at 35,000*g* for 10 min at 4°C was resuspended in the 'ATPase assay buffer.' The lutoids so obtained could be submitted to two successive osmotic shocks at 4°C, in a medium which contained 50 mM Hepes-Mes-Tris (pH 7) at a final volume of 10 ml for 200 mg fresh lutoids. After one first centrifugation at 30,000*g* for 20 min at 4°C, the membrane obtained was washed two times in the same medium and resuspended in the ATPase assay buffer.

Preparation of the Ultrafiltrated Cytosol. The first supernatant collected, defined as cytosol, was deproteinized through an Amicon PM 10 membrane which retains all the compounds of a mol wt > 10 kD, using an Amicon 8 MC concentration cell, under N_2 pressure (4 bars) at 4°C. Then, a buffered solution 500 mM Hepes-Mes-Tris (pH 7), 50 mM $MgSO_4$, 1 mM ammonium molybdate was added in the following ratio: 90% (v/v) ultrafiltrated cytosol, and 10% (v/v) buffered medium. The deproteinized and buffered cytosol constitute the incubation medium used for the ATPase assays in quasi *in vivo* conditions.

ATPase Assays. Tonoplast ATPase activity was measured in fresh intact lutoids or in purified membranes isolated from lutoids after two successive osmotic shocks. The ATPase assay buffer for ATPase activity determination was the following: 50 mM Hepes-Mes-Tris (pH 7), 300 mM mannitol, 5 mM $MgSO_4$, 0.1 mM ammonium molybdate (in order to inhibit any residual acid phosphatase activity associated with the membrane, as described by D'Auzac [10]). Assays with artificial buffer or ultrafiltrated cytosol were performed in a final volume of 2.5 ml with 10% (w/v) lutoids or membrane at a final concentration around 10 mg protein·ml^{-1}. The reaction was started by the addition of 5 mM ATP at pH 7. The incubation time was 10 min at 26°C under continuous stirring. Then, enzymic hydrolysis of ATP was stopped by addition of ice-cooled TCA at a final concentration of 0.5 mM. The ADP release was measured enzymically according to the method of Adam modified by D'Auzac (10). A control without lutoids was realized in order to determine contamination of commercial ATP by ADP and endogenous concentration of ADP.

Estimation of Δ pH. Δ pH was estimated by following the changes in the [^{14}C]methylamine level in the lutoid fraction assuming that this amine was accumulated in the most acidic space. Assays were performed in a final volume of 2.5 ml of the ATPase assay buffer described above, containing 100 μM of cold methylamine and 0.9 × 10^4 Bq of labeled methylamine. After 10 min of preincubation (time required to obtained equilibration of methylamine across the lutoid tonoplast), reactions were started by addition of ATP, 5 mM final concentration, previously adjusted to pH 7. After 10 min incubation at 26°C the ATP-dependent proton pumping was stopped by addition of ice-cold NEM, which inhibits dramatically lutoid H^+-ATPase (14), at a final concentration of 500 μM and immediate centrifugation at 30,000*g* for 5 min at 4°C. An aliquot of the supernatant was taken for determination of radioactivity, then supernatant was discarded and carefully drained off from pellet. The pellet was weighed and its radioactivity determined after addition of 500 μl of 0.1% (v/v) Triton X-100. The H^+-transport was expressed as cpm of [^{14}C]methylamine uptake for 10 min/100 mg of lutoids (as fresh weight).

Ion Exchanger. Specific trapping of cytosolic ions was achieved by incubation of 2.5 ml of cytosol, gently stirred at room temperature during 1 h with 0.5 g of washed 'Dowex,' either a strongly basic anion exchanger (OH^- form), or a strongly acidic cation exchanger (H^+ form). Ion exchangers were released by centrifugation and the treated cytosol was neutralized by addition of concentrated Mes or Tris buffers.

Protein Estimation. After alkaline solubilization 0.1 N NaOH final concentration, proteins were determined as described by Lowry et al. (21) using BSA as the standard.

RESULTS

Figure 1 shows that the ethrel stimulation of *Hevea* enhances tonoplastic ATPase activity in washed intact lutoids. This increasing activity is already significant 12 h after treatment. The maximum is reached at 36 h, then after 48 h the enzyme activity stabilizes (+100% activation). This effect is even stronger in purified lutoid tonoplast (Fig. 2), for which there is 400% activation. The maximum activation is observed 24 h after the application of the ethylene producing compound on *Hevea* bark, and is maintained during the next 48 h with a slight decrease of the observed activation. The homogeneity of the 'lutoid ATPase parameter' within the group of selected *Hevea* trees, demonstrate that the stimulating ethrel effect is highly significant. A 100% activation of the ATP-dependent H^+-pumping, estimated from the distribution of the [^{14}C]methylamine across the lutoid membrane, is observed concomitantly to the ATPase activation (Fig. 3). Preliminary experiments have demonstrated that the activation of the tonoplast-bound ATPase induced by ethrel is as transient as the ethylene-induced increase of yield, indeed 96 h after the application of stimulating treatment a 30% residual activation of both ATPase and H^+-translocating activities was observed, and no more activation after 144 h (data not shown). The *in vitro* bubbling, up to 1 ml·L^{-1} of ethylene, which approximatively corresponds to dissolved ethylene concentration of 500 μM, either into the ATPase assay buffer or in the ultrafiltrated cytosol, does not induce any activation of tonoplast-bound ATPase (data not shown). This result eliminates the possibility of a direct effect of ethylene on the ATPase. As the activation of the tonoplast-bound ATPase is observed in a totally

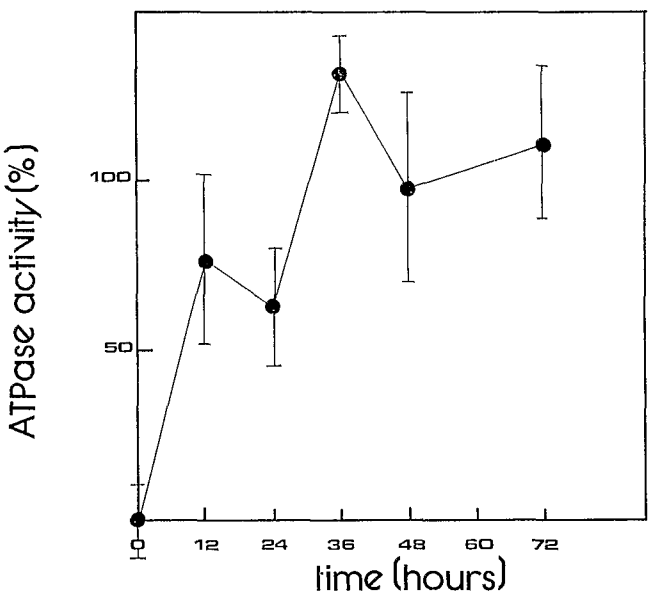

FIG. 1. Kinetics of the 'ethrel effect' on the ATPase activity of intact washed lutoids, expressed as a percentage of activation as compared to the untreated control. Each experimental point represents the mean of four determinations (duplicate assay for each tree of the group). Vertical lines represent the maximal amplitude of variation observed. The mean ATPase activity and the confidence interval ($\alpha = 0.05$) for the 12 *Hevea* trees used in this experiment were, before the ethrel treatment, 0.92 ± 0.12 μmol ATP hydrolyzed·min^{-1}·mg^{-1} protein.

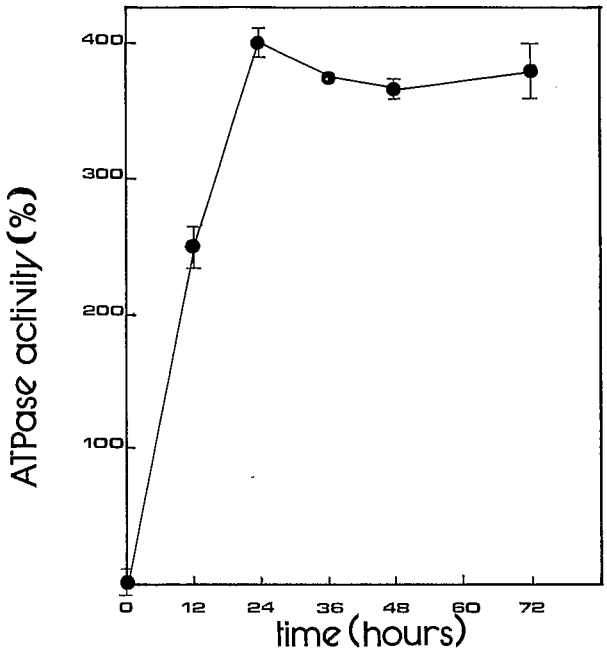

FIG. 2. Kinetics of the ethrel effect on the ATPase activity of the purified lutoid tonoplast. Results are expressed as in Figure 1. The mean ATPase activity and the confidence interval (α = 0.05) for the 12 *Hevea* trees used in this experiment were, before the ethrel treatment, 1.06 ± 0.12 μmol ATP hydrolyzed·min^{-1}·mg^{-1} protein.

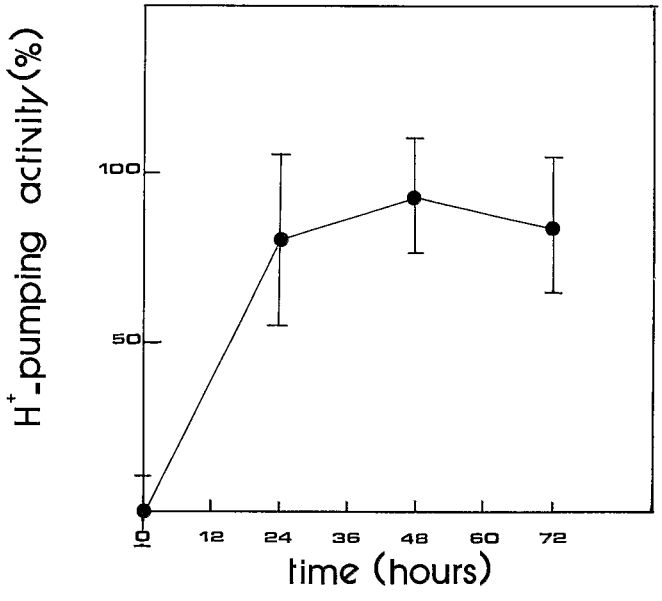

FIG. 3. Kinetics of the ethrel effect on the ATP-dependent H^+-translocating activity of intact lutoids estimated from the distribution of the [^{14}C]methylamine across the lutoid tonoplast. Results are expressed as in Figure 1.

artificial medium with washed intact lutoids and overall with purified membranes, we conclude that the lutoids conserve 'the stamp' of the stimulating effect integrated or recorded in their membrane.

In order to test the hypothesis of a 'soluble activator' present in the latex cytosol from stimulated trees, experiments involving 'crossed incubations' were conducted: intact lutoids from stimulated and nonstimulated trees are resuspended in ultrafiltrated cytosol from control or ethrel-treated trees. Table I shows that the tonoplast ATPase is activated in the ultrafiltrated cytosol, in all cases. However, the laticiferous cytosol from ethrel-treated trees shows a higher stimulating effect than the cytosol from control trees. Therefore, one or several activating compounds are present in the latex. These compounds have a low mol wt (<10,000), and are able to stimulate the ATPase activity. Ethylene induces an increase in the amount of the preexisting cytosolic activator and/or the appearance of new stimulating compounds and/or the disappearance of preexisting inhibitors.

The use of boiled cytosol as the incubation medium shows that the endogenous effector is heat-resistant; consequently, it is not a protein (Table II). Ion-specific trapping on ion exchangers shows that the endogenous activator is anionic, because the exchange of endogenous anions against hydroxyl group of either control or ethrel-treated cytosol restores the ATPase activity observed when lutoids are incubated in buffer (Table II). However, the activity measured after the anions exchange from the cytosol of ethrel-treated trees is weaker than that obtained in artificial buffer, suggesting the appearance of an inhibitory cation following the addition of ethrel. The 25% activation observed following the cation-specific trapping treatment of the ethrel-treated cytosol, confirms the appearance of an inhibitory cation after stimulation by ethrel.

Table I. *Crossed Incubations*

Tonoplast ATPase specific activities were determined in washed intact lutoids, from control and ethrel-treated trees, resuspended in an isotonic artificial buffer, or in ultrafiltrated cytosol (adjusted to pH 7), from control and stimulated *Hevea*. Experiments were done on a mixture of latex from 10 control trees and another one from 10 stimulated trees. Extraction of lutoids and preparation of ultrafiltrated cytosol were realized with this mixture as described in "*Materials and Methods*."

Lutoids	ATPase Activity		
	Isotonic buffer	Control cytosol	Ethrel-treated cytosol
	μmol·min^{-1}·mg^{-1} protein		
Control	0.38 (0)[a]	0.66 (74)	0.98 (118)
Stimulated	0.84 (121)	0.88 (132)	1.17 (207)

[a] Percent activation as compared to the control.

Table II. *Endogenous Effectors of Tonoplast-Bound ATPase*

Aliquots of cytosol (2.5 ml) were treated with ion exchangers as described in "Materials and Methods," then adjusted to pH 7, or boiled in order to determined the nature of endogenous effectors. Lutoids when then incubated with the different aliquots and the ATPase activity was assayed. Specific activity of the lutoids in ATPase assay buffer was 0.92 μmol ATP hydrolyzed · min^{-1} · mg^{-1} protein.

Condition	Specific Activity	
	Control cytosol	Ethrel-treated cytosol
	μmol·min^{-1}·mg^{-1} protein	
Control	1.23 (100)[a]	1.55 (100)
Cation exchanger	1.20 (98)	1.93 (125)
Anion exchanger	0.94 (76)	0.76 (49)
Boiling for 10 min	1.26 (102)	1.74 (112)

[a] Percentage of activities as compared to the untreated cytosol.

DISCUSSION

The reproduction of the phenomenon observed under various experimental conditions as well as its amplitude, allow to establish firmly that the application of ethrel, an ethylene producing compound, on *Hevea* bark, induces a dramatic increase of the tonoplast-bound ATPase activity, to reach a plateau 24 to 36 h after the application of the stimulating agent. The stronger effect observed with purified tonoplast could be due to the diminution of the protein content leading to an higher specific activity and/or to the absence of rate limitation by the existing ΔH^+ across the intact lutoids. This ATPase activation seems to be the result of two components: the first one would be integrated or 'stamped' in the membrane because it remains observable with purified membrane incubated in artificial buffer, the second one would be a cytosolic endogenous effector.

Ethylene does not exert any direct effect on tonoplast-bound ATPase, as demonstrated by the *in vitro* bubbling of ethylene. In the same way it has been demonstrated that ethylene has no apparent effect on the membrane potential of plant mitochondria oxidizing succinate under either phosphorylating or nonphosphorylating conditions (12). Furthermore, ethrel does not generate any significant modification in the lipid composition of the lutoid membrane (H Chrestin, unpublished results). Therefore, one possible explanation for the first component of the ethrel-induced stimulating effect, would be the induction of the tonoplast ATPase, considering that ethylene exerts multiple regulatory controls on the expression of the genetic information at both transcription and translation levels after 21 h of treatment, as demonstrated in carrot roots by Laties' group (6, 26). In the same way, Zurfluh and Guilfoyle (29) have demonstrated ethylene-induced changes in the proportion of translatable mRNA in soybean hypocotyl. Nevertheless, direct evidences for the induction of the synthesis of tonoplast-bound ATPase, consecutively to the ethrel treatment, remains to be clearly demonstrated.

The second part of the activation is ensured by an endogenous effector, always present in the cytosol of laticiferous cell, but whose stimulating effect is increased by the ethrel treatment, as demonstrated by the 'crossed incubation' experiment. Our results show that the ultrafiltrated laticiferous cytosol from ethrel-treated trees stimulates the ATPase activity more than the cytosol from control trees. Therefore, it is possible that ethylene induces a substantial increase of the amount of 'physiological activator(s);' these activator(s) may have low mol wt, are present in the fresh latex, and are probably anions. They could include malate and/or carbonate and/or chloride, which strongly stimulate the tonoplastic ATPase activity (14, 23). This activation by anions (mainly chloride) excepting nitrate, seems to be characteristic of the tonoplast-bound H^+-translocating ATPase and has been demonstrated in numerous plant materials, such as microsomal vesicles from maize coleoptile (15), oat roots (7), and soybean roots (18), and in isolated vacuoles from *Tulipa* petals (20). Considering that the chloride concentration in the cytosol from laticiferous cell is in the range 2 to 4 mM, this anion is a potential candidate for the activating effect. However, *in vitro* assay of tonoplast-bound ATPase have shown that in presence of 50 mM KCl, either with tonoplast membrane or with purified enzyme, the maximal stimulation obtained was around 50% (23). Therefore, the effect of chloride cannot account for the total activation observed consecutively to the ethrel treatment. Studies on ion pool changes in latex after stimulation by ethrel, have shown that there is no change in the malate concentration and also that there is a marked increase in the decarboxylation activity, which significantly increases the carbonate concentration within the latex (JL Jacob, personal communication), and may finally result in an activation of the ATPase. Unfortunately, we have no data about the evolution of the chloride content in latex consecutively to the ethrel treatment. Several anions could have a synergic effect in activating tonoplast ATPase.

The appearance of inhibitory cations following the ethrel treatment cannot mask the strong activation of tonoplast-bound ATPase. However, it has been observed that the overstimulation of *Hevea* with ethrel, could lead to a reduced rubber production. The enhancement of the concentration of the inhibitory cation following frequent ethrel-treatment could explain this phenomenon.

In conclusion, the ethrel-induced activation of the tonoplast-bound ATPase seems the result of a dual mechanism. But whatever the molecular mechanisms for this dramatic ATPase activation by ethylene, a direct consequence of these phenomena could be a noticeable *in situ* stimulation of the tonoplast ATP-dependent proton pump as demonstrated *in vitro*. This stimulation could explain the acidification of the vacuo-lysosomal compartment and the consequent alkalinization of the cytosol, which have been observed in the latex of *Hevea* trees after the application on *Hevea* bark of ethylene-producing compounds (3, 5) and which results in an enhanced rubber production.

LITERATURE CITED

1. Apelbaum A, C Vinkler, E Sfakiotakis, DR Rilley 1984 Increased mitochondrial DNA and RNA polymerase activity in ethylene-treated potato tubers. Plant Physiol 76: 461–464
2. Boller T, A Gehri, F Mauch, U Vegeli 1983 Chitinase in bean leaves: induction by ethylene, purification, properties and possible function. Planta 157: 22–31
3. Brzozowska-Hanower J, H Cretin, P Hanower, P Michel 1979 Variations de pH entre compartiments vacuolaire et cytoplasmique au sein du latex d'*Hevea brasiliensis*. Influence saisonnière et action du traitement par l'éthrel générateur d'éthylène. Répercussion sur la production et l'apparition d'encoches sèches. Physiol Veg 17: 851–867
4. Chalutz E 1973 Ethylene-induced phenylalanine ammonia-lyase activity in carrot roots. Plant Physiol 51: 1033–1036
5. Chrestin H, X Gidrol, B Marin, JL Jacob, J D'Auzac 1984 Role of the lutoidic tonoplast in the control of the cytosolic homeostasis within the laticiferous cells of *Hevea*. Z Pflanzenphysiol 114: 269–277
6. Christofferson RE, GG Laties 1982 Ethylene regulation of gene expression in carrots. Proc Natl Acad Sci USA 79: 4060–4063
7. Churchill KA, H Sze 1984 Anion-sensitive, H^+-pumping ATPase of oat roots. Direct effects of Cl^-, NO_3^-, and disulfonic stilbene. Plant Physiol 76: 490–497
8. Cretin H 1981 The proton gradient across the vacuo-lysosomal membrane of lutoids from the latex of *Hevea brasiliensis*. I Further evidence for a proton-translocating ATPase on the vacuo-lysosomal membrane of intact lutoids. J Membr Biol 65: 174–184
9. Cretin H 1982 Efflux transtonoplastique de protons lors du fonctionnement d'un système transporteur d'élèctrons (la NADH-cytochrome c réductase) membranaire des vacuolysosomes du latex d'*Hevea brasiliensis*. C R Acad Sci Paris 296: 137–142
10. D'Auzac J 1975 Caractérisation d'une ATPase membranaire en présence d'une phosphatase acide dans les lutoides du latex d'*Hevea brasiliensis*. Phytochemistry 14: 671–675
11. D'Auzac J, D Ribailler 1969 L'éthylène, nouvel agent stimulant la production du latex chez l'*Hevea brasiliensis*. C R Acad Sci Paris 268: 3046–3049
12. Djebar MR, F Moreau 1987 Effects of ethylene on membrane potential and phosphorylating properties of isolated plant mitochondria. Plant Physiol Biochem 25: 111–116
13. Eschbach JM, D Roussel, H Van de Sype, JL Jacob, J D'Auzac 1984 Relationships between yield and clonal physiological characteristics of latex from *Hevea brasiliensis*. Physiol Veg 22: 295–304
14. Gidrol X, B Marin, H Chrestin, J D'Auzac 1985 The functioning of tonoplast H^+-translocating ATPase from *Hevea* latex in physiological conditions. *In* B Marin, ed, Biochemistry and Function of Vacuolar ATPase in Fungi and Plants. Springer-Verlag, North-Holland, pp 151–163
15. Hager A, M Helme 1981 Properties of an ATP-fueled, Cl^--dependent proton pump localized in membranes of microsomal vesicles from maize coleoptiles. Z Naturforsch 36c: 997–1008
16. Jacob JL 1970 Particularités de la glycolyse et de sa régulation su sein du latex d'*Hevea brasiliensis*. Physiol Veg 8: 395–411
17. Lacrotte R, H Van de Sype, H Chrestin 1985 Influence de l'éthylène sur l'utilisation du saccharose exogène par les laticifères d'*Hevea brasiliensis*: proposition d'un mécanisme d'action. Physiol Veg 23: 187–198
18. Lew RR, RM Spanswick 1985 Characterization of anion effects on the nitrate-sensitive ATP-dependent proton pumping activity of soybean (*Glycine max* L.) seedling root microsomes. Plant Physiol 77: 352–357

19. LIEBERMAN M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
20. LIN W, GJ WAGNER, HW SIEGELMAN, G HIND 1977 Membrane-bound ATPase of intact vacuoles and tonoplasts isolated from mature plant tissue. Biochim Biophys Acta 465: 110–117
21. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275
22. MARIN B, M MARIN-LANZA, E KOMOR 1981 The protonmotive difference across the vacuo-lysosomal membrane of *Hevea brasiliensis* (rubber tree) and its modification by a membrane-bound adenosine triphosphatase. Biochem J 198: 365–372
23. MARIN B, X GIDROL 1985 Chloride-ion stimulation of the tonoplast H^+-translocating ATPase from *Hevea brasiliensis* (rubber tree) latex. Biochem J 226: 85–94
24. MARIN B, J PREISSER, E KOMOR 1985 Solubilization and purification of the ATPase from the tonoplast of *Hevea*. Eur J Biochem 151: 131–140
25. MARIN B, X GIDROL, H CHRESTIN, J D'AUZAC 1986 The tonoplast proton-translocating ATPase of higher plants as a third class of proton-pumps. Biochimie 68: 1263–1277
26. NICHOLS SE, GG LATIES 1984 Ethylene-regulated gene transcription in carrot roots. Plant Molec Biol 3: 393–401
27. PUJARNISCLE S 1968 Caractère lysosomal des lutoïdes du latex d'*Hevea brasiliensis* Mul-Arg. Physiol Veg 6: 27–46
28. THEOLOGIS A, GG LATIES 1982 Potentiating effect of pure oxygen on the enhancement of respiration by ethylene in plant storage organs: a comparative study. Plant Physiol 69: 1031–1035
29. ZURFLUH LL, TJ GUILFOYLE 1982 Auxin- and ethylene-induced changes in the population of translatable messenger RNA in basal section and intact soybean hypocotyl. Plant Physiol 69: 338–340