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# Role of the Lutoidic Tonoplast in the Control of the Cytosolic Homeostasis within the Laticiferous Cells of *Hevea*

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## Summary

Lutoids, the vacuo-lysosomes of the *Hevea* latex cells, compartmentalize, *in vivo*, numerous ions such as $H^+$, $Mg^{++}$, $Ca^{++}$, Pi, citrate, some of them strongly toxic for the cytosolic metabolism. Evidence is given for the correlation of the *in vivo* compartimentation of some of these ions inside the lutoids with the latex production by *Hevea*.

Two opposing $H^+$ pumps were localized on the lutoidic tonoplast; the one is a $Mg^{++}$-dependent ATPase, the other a NADH-consuming redox system (cytochrome c: artificial acceptor). The functioning of these $H^+$ pumps may account for a major part of the transtonoplastic $\Delta pH$ variations, and therefore the cytosolic pH control, which probably regulates the highly pH-dependent latex metabolism.

The resulting proton-motive force energizes the accumulation and compartmentation of the inhibiting ions inside the lutoïds, and ensures the control of the «detoxification» and ionic equilibrium of the cytoplasm of the laticiferous cells.

Treatment of *Hevea* bark with ethrel, an ethylene generator which «stimulates» latex production, induces an increase in the $H^+$-pumping ATPase activity, resulting in the activation of the metabolism in the latex cells.

All the results reviewed lead us to propose that the lutoids play a double role as a «biophysical pH-STAT and a «detoxicating trap», thus controlling the cytosolic homeostasis.

*Key words: Hevea brasiliensis, laticifers, lutoids, protons pumps, ATPase, NAD(P)H-oxidase, tonoplast.*

The latex from *Hevea brasiliensis* is a fluid cytoplasm which is expelled from wounded laticiferous «vessels» (articulated, anastomosed cells) (Archer et al., 1963). It contains a vacuolar compartment: the so-called «lutoids», consisting of micro-vacuoles with lysosomal characteristics which can be easily isolated and purified by simple differential centrifugation (Pujarniscle, 1968; Ribaillier et al., 1971; d'Auzac et al., 1982).

*) Officially changed name Chréstin Hervé from Crétin Hervé.

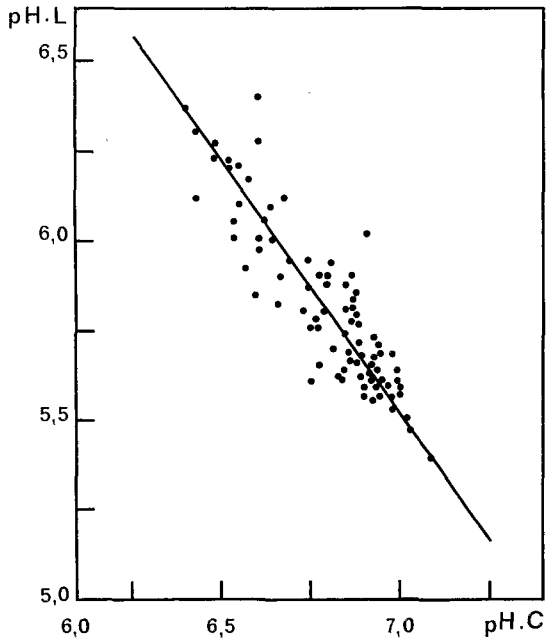


Fig. 1: Correlation between the cytoplasmic pH (pH C) and the «lutoidic» (vacuolar) pH (pH.L) in the freshly collected latex from rubber trees (measured by mean of a pH electrode).

Table 1: Table of the correlation coefficients linking the latex production (g dry rubber/tapping/tree), the latex cytosolic pH, the transtonoplastic pH gradient, the latex cytosolic and vacuolar citrate concentrations (mM) and the resulting transtonoplastisc citrate gradient [data obtained from freshly collected latex of 56 rubber trees (***): very high significance; (**): high significance].

| | Latex Production | Vacuolar (citrate) | Cytosolic (Citrate) | (Citrate) gradient | Cytosolic pH | transtonoplastic pH gradient |
|---|---|---|---|---|---|---|
| Latex Production | 1 | +0.562 *** | −0.768 *** | +0.755 *** | +0.894 *** | +0.822 *** |
| Vacuolar (citrate) | | 1 | −0.369 ** | +0.778 *** | +0.675 *** | +0.640 *** |
| Cytosolic (citrate) | | | 1 | −0.678 *** | −0.705 *** | −0.752 *** |
| Citrate Gradient | | | | 1 | +0.765 *** | +0.800 *** |
| Cytosolic pH | | | | | 1 | +0.935 *** |
| Transtonoplastic pH gradient | | | | | | 1 |

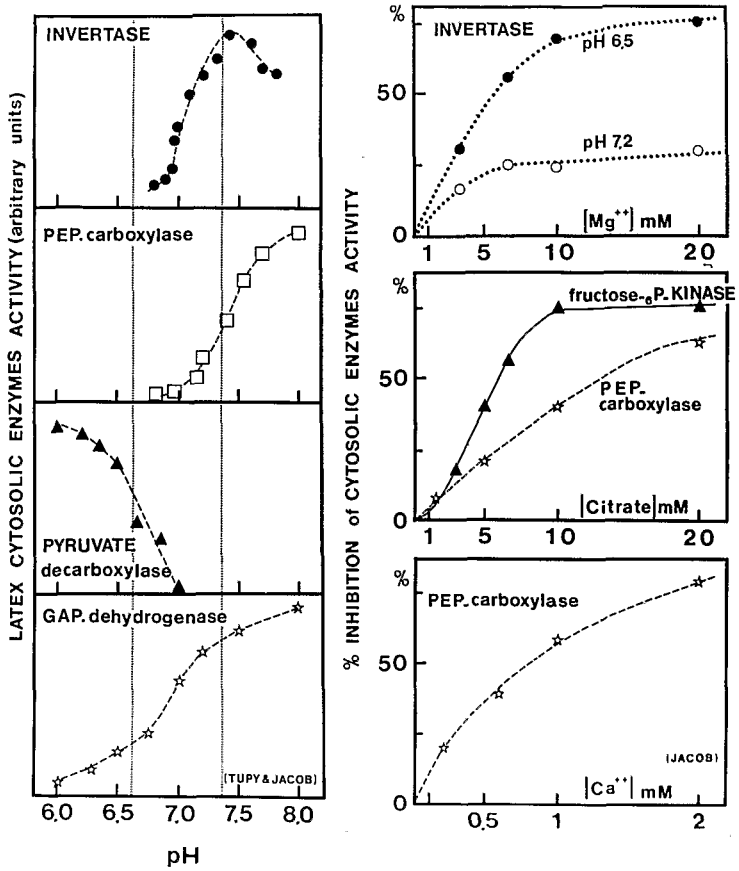


Fig. 2: a) Dependence on pH of invertase, PEP-carboxylase, pyruvate decarboxylase and glyceraldehyde 3-phosphate dehydrogenase from the latex cytosol (activities are expressed in arbitrary units) as measured in ultrafiltered latex cytosol. – b) Inhibition of latex-cytosolic invertase by $Mg^{++}$, phospho-fructo kinase and PEP carboxylase by citrate, and PEP carboxylase by $Ca^{++}$, measured in artificial buffers.

Production of latex reflects the intensity of metabolism within these specialized cells. It must be sufficient to regenerate and compensate for the loss of latex upon each tapping (generally twice a week).

Rubber production has been shown to be correlated positively to the pH of the cytosol of the latex cell and negatively with the intravacuolar pH (Coupé and Lambert, 1977; Brzozowska-Hanower et al., 1979). Further, we demonstrated a highly significant inverse relationship between the pH of the cytosolic compartment and the changes in intravacuolar pH (lutoidic pH), suggesting the existence of some vec-

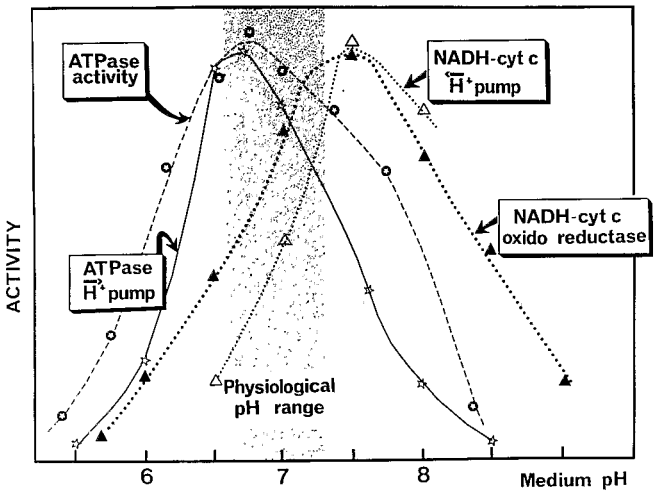


Fig. 3: Dependence on pH of the tonoplastic ATPase and NADH c cytochrome oxidoreductase activities, and of their proton pumping efficiency, measured in buffered ultrafiltered cytosol from *Hevea* latex.

torial $H^+$ fluxes at the level of the lutoidic tonoplast (Fig. 1) Brzozowska-Hanower et al., 1979).

Multivariate analysis shows that the latex from high yielding rubber-trees is not only characterized by a slightly alkaline cytosolic pH and a high transtonoplastic $H^+$ gradient, but also by a pronounced accumulation of citrate in the vacuoles, resulting in a high transtonoplastic citrate gradient, i.e. a low citrate concentration in the cytosol (Table 1).

These relationships could be explained satisfactorily by the extreme pH sensitivity (physiological pH range) of numerous key enzymes of cytosolic metabolism and their inhibition by some ions, such as citrate, $Ca^{++}$, $Mg^{++}$, and $Cu^{++}$, at physiological concentrations (for example see Fig. 2 a–b) (Jacob and d'Auzac 1967, 1969, 1972; d'Auzac and Jacob, 1969; Tupy, 1969, 1973; Jacob et al., 1979).

The transtonoplastic $H^+$ fluxes, the cytosolic as well as the vacuolar pH changes, are shown to be under the control of two opposing $H^+$ translocating systems, located at the level of the lutoidic tonoplast (Crétin et al., 1982).

The first is a tonoplastic ATPase dependent on $Mg^{++}$ (d'Auzac, 1975 and 1977), which catalyses $H^+$ influx into the vacuole causing cytosolic alkalinization and an increase in transtonoplastic pH gradient (Marin et al., 1981; Crétin, 1982; Crétin et al., 1982; Marin and Blasco, 1982).

The other is a tonoplastic electron-transporting system, NADH-cytochrome (artificial acceptor) oxido-reductase, and may be the same as discovered by Moreau et al. (1975). This redox-chain induces $H^+$-efflux, followed by cytosolic acidification, and a collapse of the transtonoplastic pH gradient (Crétin, 1983).

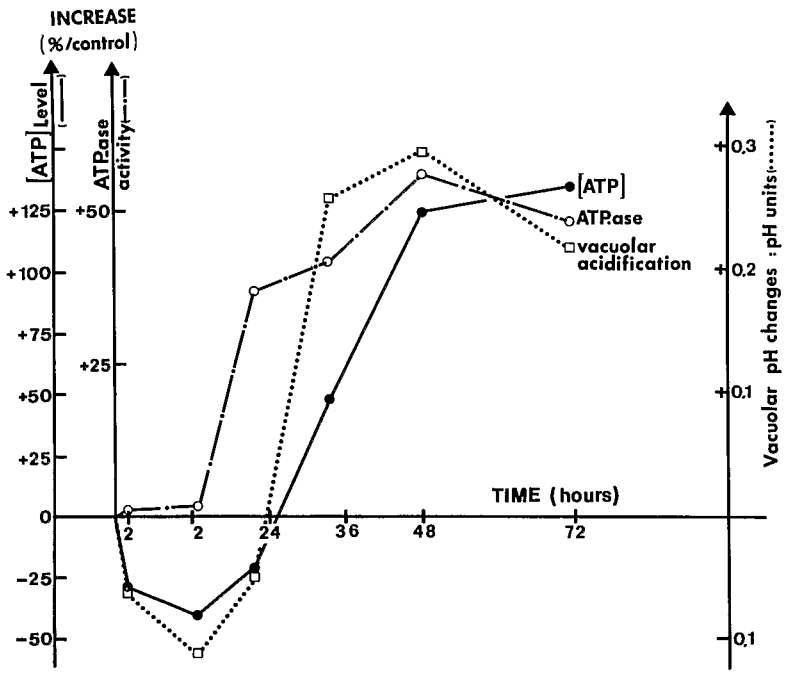


Fig. 4: Kinetics of the Ethrel effects on the tonoplastic ATPase potential activity (– · – · – · –), the cytosolic ATP level (———) as expressed in % variations compared with the control, and on the vacuolar acidification ( · · · ), in the latex from ethrel treated *Hevea* bark (base line = control).

Plotting the activity of these two $H^+$ pumps as a function of the pH of the medium shows that the ATPase remains at its maximal potential activity over the physiological range of pH, while the tonoplastic $e^-$ transport chain, being pH sensitive (same pH range), becomes more efficient at a slightly alkaline pH (Fig. 3). This suggests that an excessive ATPase-induced cytosolic alkalinization will be counteracted by the activation of the NADH dependent $H^+$ efflux.

Moreover, these two $H^+$-pumping systems, which up to now have been characterized in totally artificial isotonic buffers, have recently been shown to function at least as well in «quasi *in vivo*» media (fresh latex cytosol deproteinized by ultrafiltration on AMICON molecular filter PM 10) (Crétin et al., 1983) suggesting that these pumps may indeed function *in vivo*.

Treatment of *Hevea* bark with ethrel, an ethylene generator which «stimulates» latex production (d'Auzac and Ribaillier, 1969), induces an increase in the activity of the $H^+$-pumping ATPase, probably owing to activation of specific protein synthesis at the tonoplast level. The major part of this ATPase activation results in fact from a

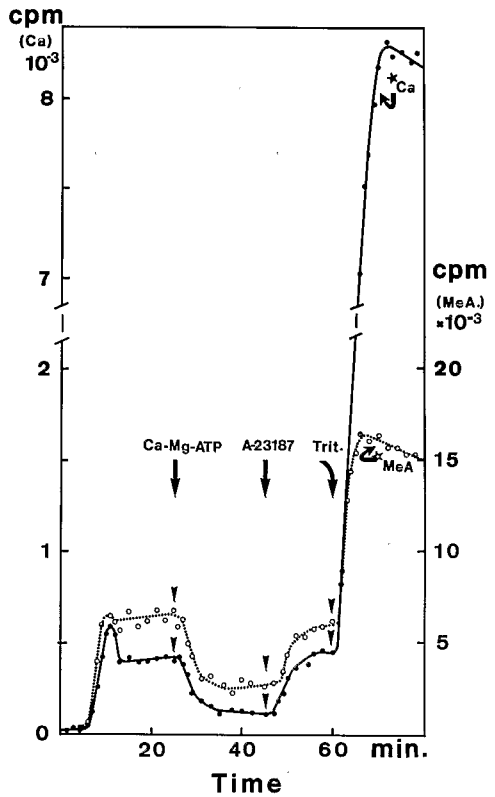


Fig. 5: Effects of addition of ($Ca^{++}$ + $Mg^{++}$) and the divalent ionophore A-23187, on the $^{45}Ca^{++}$ (——) and methylamine fluxes ( · · · ) across the lutoidic tonoplast. Lutoids were loaded until equilibrium, with $^{45}$Calcium or $^{14}$C-methylamine (MeA) at pH 7.0; the $Ca^{++}$ and MeA (pH probe) changes were continuously monitored in the external medium by the flow dialysis technique (Crétin, 1982). Effectors were added as indicated by the arrows. At the end of the experiment lutoids were lysed by triton (0.5 %).

great increase in the concentration of the substrate ATP (Fig. 4), and the appearance of some low molecular weight activators in the cytosol (Crétin et al., 1983). This activation of ATPase induces a great increase of transtonoplastic ΔpH and thus a marked cytosolic alkalinization (Tupy, 1973; Coupé et al., 1976; Coupé, 1977; Brzozowska-Hanower et al., 1979) resulting in the activation of metabolism in the laticifers.

Citrate, a potent inhibitor of numerous key enzymes of the cytosolic metabolism when present in excess concentrations (Jacob and d'Auzac, 1967; Jacob, 1970; Jacob et al., 1979) in the cytosol ($\geq$ 7 mM), is compartmentalized *in vivo* inside the latex vacuoles (40 to 80 mM in the lutoids) (Ribaillier et al., 1971; Chréstin, unpublished).

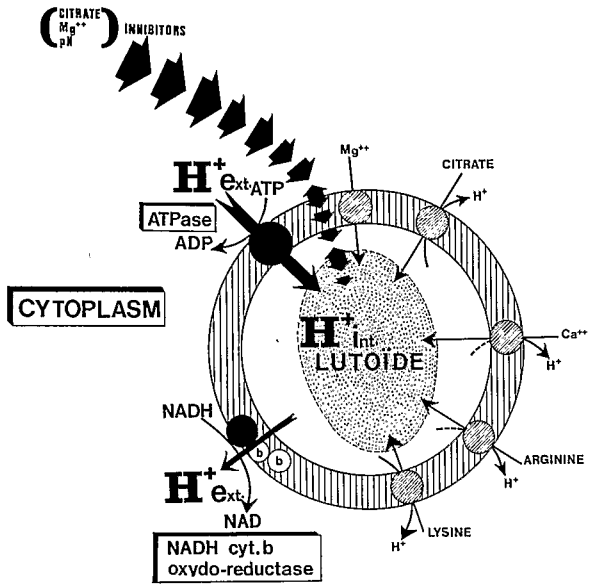


Fig. 6: The dual role of the lutoidic tonoplast in the control of the cytosolic homeostasis in *Hevea* latex: as a «biophysical pH-STAT» and a «detoxicating TRAP» compartmentalizing inhibitory ions of the cytosolic metabolism. The figure shows the present state of knowledge about the energization of transtonoplastic $H^+$ fluxes and transport of solutes in the lutoids. The tonoplast ATPase and «redox system» function as opposing proton pumps. Data obtained from citrate, lysine and $Ca^{++}$ fluxes across the lutoidic tonoplast agree with mechanisms of solutes/proton(s) antiporter. The existence of two kinetic pools for accumulated solutes is proposed.

*In vivo*, the lutoïds accumulate exogenous citrate against a steep concentration gradient (d'Auzac and Lioret, 1974; Montardy and Lambert, 1977), and any amplification of the transtonoplastic $H^+$-gradient leads to the transport of citrate into the vacuolar space (Marin, Marin et al., 1981). Mg-ATP is efficient in the energetization of citrate accumulation. This ATP-energized influx of citrate is inhibited by protonophores (Marin, 1981; Marin et al., 1981; Chréstin, unpublished). However attempts to provoke efflux of the accumulated citrate, by collapsing either or both the transtonoplastic pH and the potential gradients of *intact lutoids, in vivo,* were ineffective so far (Montardy and Lambert, 1977; Chréstin, unpublished).

We therefore suggest that the lutoids behave as a «non-reversible detoxifying trap» and prevent the inhibition of the cytosolic metabolism by excess of citrate.

Although $Ca^{2+}$ is necessary for optimal activity of metabolism, if present in the cytosol at concentrations $>0.5$ mM it inhibits some cytosolic key enzymes (Jacob et al., 1979). *In vivo,* $Ca^{++}$ ions are normally compartmented (up to 2.5 mM) inside the lutoidic vacuolar space (Ribaillier et al., 1971).

*In vitro,* the lutoids strongly accumulate $Ca^{++}$ against a steep concentration gradient, and $Ca^{++}$ fluxes always follow the $H^+$ fluxes i.e. the changes of the transtonoplastic pH gradient (Chréstin, unpublished; Marin et al., 1982). Mg-ATP is efficient in energizing $Ca^{++}$-accumulation in the lutoids (Fig. 5). Which is inhibited by protonophores. Part of the (free?) $Ca^{++}$ accumulated is released into the external medium if lutoids are treated with divalent cation ionophores (Fig. 5), protonophores, acidified medium, or if the NADH-dependent $H^+$-efflux pump is allowed to work (Crétin, unpublished results).

Consistent with the functioning of the two opposing $H^+$-pumps at the tonoplast, we propose that the vacuolar compartment of the latex cells (d'Auzac et al., 1982) plays a double role as a «biophysical pH-STAT» and a «detoxicating trap», thus controlling homoeostasis in the cytosol and favouring active metabolism within the cells, thus resulting in high latex (rubber) production (Fig. 6).

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