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Structural and functional studies at the plasma membrane during flowering induction of Spinacia oleracea leaves

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ABSTRACT

BELLAMINE, J. & H. GREPPIN (1996). Structural and functional studies at the plasma membrane during flowering induction of Spinacia oleracea leaves. *Saussurea* 27: 41-47. In English, English and French abstracts.

Structural analysis by infrared spectroscopy of the purified plasma membrane from leaves of the long-day plant, *Spinacia oleracea* (cv L. Nobel), suggested a change in the phospholipid fatty acids during the transition from vegetative to induced state. This had been demonstrated further by gas chromatography analysis of the fatty acids, suggesting a decrease in the fluidity of the plasma membrane and could explain changes in the Km_{app} of the ATP-dependent H^+pumping activity of the plasma membrane and in the kinetics of the inhibition by vanadate of the enzyme after flowering induction. *In vitro* incubation of the plasma membrane in the presence of increased concentrations of auxin (indole 3-acetic acid), showed a decrease in the sensitivity to auxin of the H^+ATPase activity, which could be associated to floral induction. Some points of evidence suggested that the sensitivity change could be related to auxin level change in leaves, and could involve the phosphatidylinositol-cycle and calcium ions.

RÉSUMÉ

BELLAMINE, J. & H. GREPPIN (1996). Etudes structurelles et fonctionnelles de la membrane plasmique durant l'induction florale des feuilles de Spinacia oleracea. *Saussurea* 27: 41-47. En anglais, résumés anglais et français.

L'analyse structurelle par spectroscopie infrarouge de la membrane plasmique purifiée à partir des feuilles d'une plante de jour long, *Spinacia oleracea* (cv L. Nobel), a suggéré un changement de la composition des groupements acyls des phospholipides durant la transition de l'état végétatif à l'état induit. Ceci a été démontré plus tard par l'analyse par chromatographie en phase gazeuse des acides gras et suggère une diminution de la fluidité membranaire. Ceci pourrait expliquer les changements du Km_{app} de l'activité H^+ATPase de la membrane plasmique et de la cinétique d'inhibition par le vanadate de cette activité, après induction florale. L'incubation *in vitro* de la membrane plasmique, en présence de concentrations croissantes d'auxine (acide 3-indole acétique), a mis en évidence une diminution de la sensibilité à l'auxine de l'activité H^+ATPase qui pourrait être associée à l'induction florale. Quelques points d'évidence suggèrent que le changement de la sensibilité serait relié à un changement du taux en auxine dans les feuilles et pourrait impliquer le cycle du phosphatidylinositol et les ions calcium.

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Introduction

The transition from vegetative to reproductive development is a crucial phase in the life cycle of higher plants. This suggests an understanding of the physiological, biochemical and molecular aspects of reproductive development. Although a great deal of descriptive information exists for many species about the influence of environmental factors on reproductive development, the knowledge of the mechanisms by which the floral transition takes place is almost totally lacking.

In photoperiodically sensitive plants, the site of perception of daylenght is the leaf, whereas the apex is where the morphological change occurs. This suggests that some message is transferred from the leaf to the apex, causing the transition to flower formation. This signal is termed the floral stimulus, florigen, or flower hormone, the nature of which is still unknown. Further evidence for the existence of the floral stimulus was indirectly derived from grafting experiments.

Studies underwent on spinach demonstrated the possibility to induce or to inhibit the flowering by differents chemical and physical ways. The effect of these treatments has been confirmed by ultrastructural analysis of the apical meristem (AUDERSET & al., 1986). The plant development is controlled both by exogenous and endogenous signals and by genetic factors. The mechanisms involved in the recognition of these different signals and their target are still not well understood. Among the possible targets particular attention was given to the plasma membrane of leaf cells, which could be involved in the perception, transduction and transport of environmental signals into the plant and leading to evocation of apices. From this point of view, the plasma membrane could be taken as an interface for the integration of developmental factors. This hypothesis suggested a systematic study, during the early phase of the photoperiodic induction, of biochemical and biophysical properties of the plasma membrane. The obtained experimental results indicated that this photoinduction was accompanied by a thickening and by qualitative and quantitative modifications in sterols content of the plasmalemma. These modifications were first observed in leaf, early during induction (critical photoperiod: 12 h of continuous light), and just after in the apex (CRESPI & al., 1989; CRÈVECOEUR & al., 1991; GREPPIN & al., 1995).

We have investigated the changes of both structural and functional properties of the plasma membrane from leaves of the long-day plant spinach, *Spinacia oleracea* (cv L. Nobel), during the transition from vegetative to induced state, by means of infrared spectroscopy (FTIR) analysis and the study of the *in vitro* sensitivity to auxin of the plasma membrane H^+ATPase. The plasma membrane vesicles were purified by phase partition and characterized by biochemical markers.

Structural analysis of the plasma membrane of spinach leaves during the transition to flowering

Plasma membrane vesicles were prepared from spinach leaves in vegetative state and induced one either by 24 h of continuous light or by gibberellic acid treatments in short days. The analysis by infrared spectroscopy of the purified plasma membrane led to characteristic spectra (BELLAMINE & al., 1993a). Since absorption spectra obtained contain vibration bands of different plasma membrane components (proteins, lipids, polysaccharides), we expected a very complex spectrum due to the number of bands,

peak positions, half width, and relative peak intensities. These factors led to overlapping bands, whose resolution can be enhanced by calculating, from original spectra, a second derivative using IRDM (NAUMANN & al., 1988). The second derivative spectra were then divided into several windows depending on the vibration band of a given chemical function, digitized and measured for similarities using Pearson's product moment correlation coefficient. The main differences were observed for the 1800-1700 cm^{-1} and 1200-1090 cm^{-1} windows, corresponding to an ester chemical function. It seems that the flowering induction of spinach plants either by light or by gibberellic acid modified an ester function of one or several membranous structures. The main membranous structure which could be suspected and containing an ester function is the phospholipid.

Another difference observed concerned the 1700-1500 cm^{-1} window which contains a characteristic band of C = O stretching vibration of amide I and II of proteins. This difference could reflect not only a quantitative modification of the proteins but also a structural modification of the proteins into the plasma membrane.

Fatty acid methyl esters were prepared from plasma membrane phospholipids of vegetative plants and induced ones by continuous light (24 h or 48 h) or by gibberellic acid treatments in short days (BELLAMINE & al., 1994). The saturated to unsaturated phospholipid fatty acids ratio was kept constant after all treatments comparatively to untreated plants (vegetative plants). However, the C18:1/C18:2 phospholipid fatty acids ratio was increased after light induction of 24 h and was stable after 48 h of continuous light. The same changes were observed after gibberellic acid treatments in short days, suggesting that the increase of the fatty acids ratio is associated to flowering induction.

The increase in the C18:1/C18:2 fatty acids ratio after flowering induction could influence the fluidity (decrease) of the plasma membrane and then the proteins conformation and enzymatic activities. There is more and more evidence to believe that lipid components are important in the regulation of the plasma membrane H^+ATPase activity (COCUCCI & MARRE, 1984; KASAMO & NOUCHI, 1987; PALMGREN & al., 1988; PEDCHENKO & al., 1990; SCHERER & ANDRÉ, 1993). This could be illustrated in spinach plants by the observed effect on the plasma membrane ATPase activity of some added phospholipids (BELLAMINE & al., 1994; BELLAMINE & GREPPIN, 1996a). The change in membrane fluidity as evidenced by the infrared spectroscopy and phospholipid fatty acids analyses could explain the changes in the Km_{app} of the ATP-dependent H^+pumping activity and in the kinetics of the inhibition by vanadate of the enzyme after flowering induction (BELLAMINE & al., 1994).

Proton pump and auxin sensitivity changes during the flowering induction

The effect of auxin on proton extrusion by plant cells is well known (JACOBS & TAIZ, 1980; BATES & GOLDSMITH, 1983). The obtained data with the purified spinach plasma membrane showed that indole 3-acetic acid (IAA) was able to stimulate the proton translocation by acting directly on the membrane. Such an *in vitro* effect of IAA was already described for other plant species (MASSON & al., 1994). The stage of development of the plant and the treatments that it could have received before plasma membrane extraction, has an important influence on the sensitivity of the H^+ ATPase to IAA.

The sensitivity of membrane vesicles was measured in the presence of increased IAA concentrations, using membranes prepared from short day (SD) grown spinach and

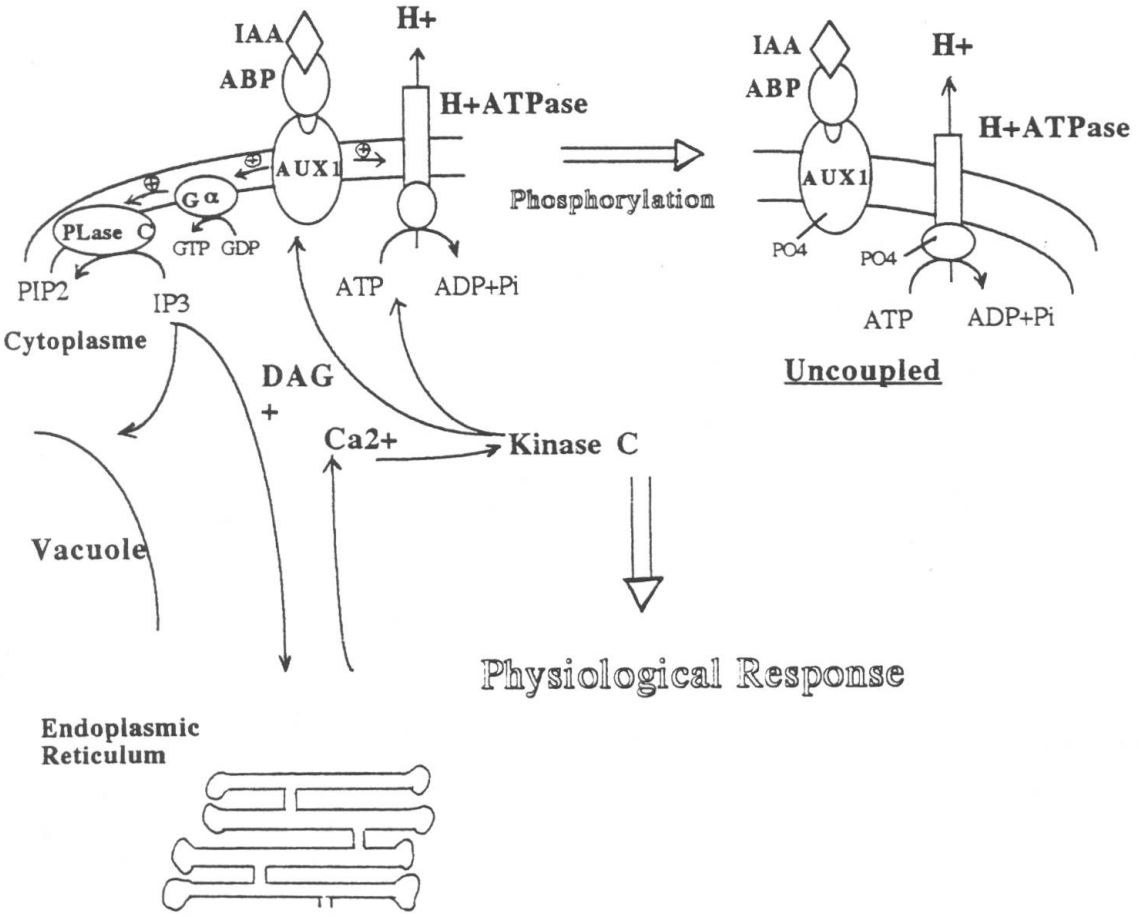
similar plants submitted to different periods of continuous light (BELLAMINE & al., 1993 b). The incubation of vesicles from vegetative leaves, in the presence of IAA, exhibited a maximum H^+ accumulation at nanomolar concentrations. Higher IAA concentrations were less active or, even, slightly inhibitory. A similar sensitivity was exhibited by the plasma membrane vesicles prepared from SD grown spinach exposed to a photoperiod of 11 hours.

Extending this light period to 13 hours induced the appearance of a second peak of sensitivity with a maximum effect at one micromolar IAA. This change was observed just around the critical photoperiod which is, for spinach, about 12 hours of continuous light (GREPPIN & al., 1991). The plasmalemma of plants exposed to longer periods of light (24 and 48 hours) exhibited also two peaks of IAA effect. This observation can be compared with the effect of IAA on the elongation of spinach petiole segments reported previously by GASPAR & al. (1985). Petiole segments from plant subjected to 24 hours light period were shown to exhibit an elongation in presence of high IAA concentrations, which is much larger than the elongation of petioles from SD grown spinach. On the other hand a very little effect could be detected with a weak acid (same pKa as IAA) devoid of hormonal activity such as acetic acid. This weak stimulation of the H^+ transport activity by acetic acid was increased, as a function of its concentration, in a manner that it could not be assigned to an auxin effect.

Exposing such SD grown (vegetative) plants to various treatments known to bring about the induction to flowering (gibberellic acid treatments in short days, displaced short day, extended growth in short days) triggered also the appearance of the second peak of ATPase activation with a maximum effect of IAA at a micromolar concentration. This established the possible relation between floral induction of spinach and the change observed in the sensitivity of H^+ transport activity to IAA. The two coexisting sensitivities – high and low – in the same membrane preparation suggest the existence of two different populations of H^+ATPases or of two mechanisms of regulation.

Auxin (IAA) promoted growth of spinach petiole segments as reported by GASPAR & al. (1985) and BELLAMINE & GREPPIN (1996b). It seemed that an extended exposure of the spinach plants to exogenous auxin led to a decrease in the sensitivity of the plasma membrane H^+ATPase to that hormone in similar manner as did light induction. This desensitization phenomenon is similar to that known in animal cells in which this could be due either to a decrease in the hormone receptor level at the cell surface (homologous desensitization) (CLARK & al., 1985) or to an uncoupling of the hormone-receptor complex to its effector system by phosphorylation (heterologous desensitization) (BRIGGS & al., 1983).

The mechanism by which the exogenous auxin leads to different cell reponses is not clearly known until now, although the auxin could activate the phosphorylation of cell proteins (POOVAIAH & al. 1988) using the PIP_2 pathway as a signal transducer system (ZOCCHI, 1990) via the activation of the G protein (ZAINA & al. 1990). In the case of spinach, the activities of both phospholipase C and G proteins have been identified and characterized on the plasmalemma of leaf cells (CRESPI & al., 1993; CRESPI & al., 1996). Auxin sensitivity in the assay medium of the H^+ATPase activity of the purified plasma membrane from leaves is reported to be influenced by a change in light period as needed for flower induction (BELLAMINE & al., 1993 b). Treatments of spinach plants, prior to membrane preparation, with substances that are supposed to affect calcium homeostasis (EGTA, A_{23187}, verapamil, and $LaCl_3$) or the phosphatidylinositol pathway (LiCl), suggested that the signalling change leading to the responses



Scheme 1. – Schematic representation of the mechanism of IAA action in the desensitization of the plasma membrane H^+ATPase of spinach leaves. ABP: auxin binding protein; AUX1: transmembrane docking protein.

involves the phosphatidylinositol-cycle and calcium ions (BELLAMINE & GREPPIN, 1996c).

Conclusion

Structural and functional modifications observed at the plasma membrane level could be associated to the induction to flowering time period in spinach plants as shown for other day-neutral and long-day plants (MASSON & al., 1994). Data obtained in tobacco and spinach plants (SANTONI & al., 1993; BELLAMINE & GREPPIN, 1996b) suggested that plasma membrane sensitivity changes evidenced during floral induction could be related to auxin concentration changes in leaves (pulse). In addition, it has been reported that floral induction coincides with a high level of endogenous auxin in some plant species (GASPAR & al., 1985).

Unfortunately, no significative changes of the polypeptide composition of the plasma membrane were observed, using our method, during floral induction in spinach leaves (LEFORT & al., 1991). As the first step of the acquisition of the floral state in leaves could not be attributed to changes in new gene expression until now

(BALET-BURON & GREPPIN, 1977), it was worth looking for quantitative and functional changes. From this point of view, the loss of sensitivity of proton pumping towards IAA, apparently associated with floral induction, could give a clue to understand the leaf process leading to flowering in relation with the plasma membrane signalling.

Scheme 1 represents a speculative cell surface events in the auxin signalling pathway. Light induction of spinach leaves and the subsequent increase in the endogenous auxin level seemed to use the phosphatidylinositol transduction pathway and the Ca^{2+} ions as a second messenger to activate a regulatory system leading to the change in the sensitivity to IAA of the plasma membrane H^+ATPase activity. A mechanism of protein phosphorylation of the H^+ATPase and/or AUX1, mediated by a protein kinase C Ca^{2+} dependent, an activated regulatory system, would lead to an uncoupling of the complexe auxin-auxin binding protein to the proton pump and then to the observed desensitization of the H^+ATPase activity to IAA, in similar manner as the heterologous desensitization in animal cells (BRIGGS & al., 1983).

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