Spatial organization of tobacco cell plasma membrane : characterization, and modulation upon elicitation

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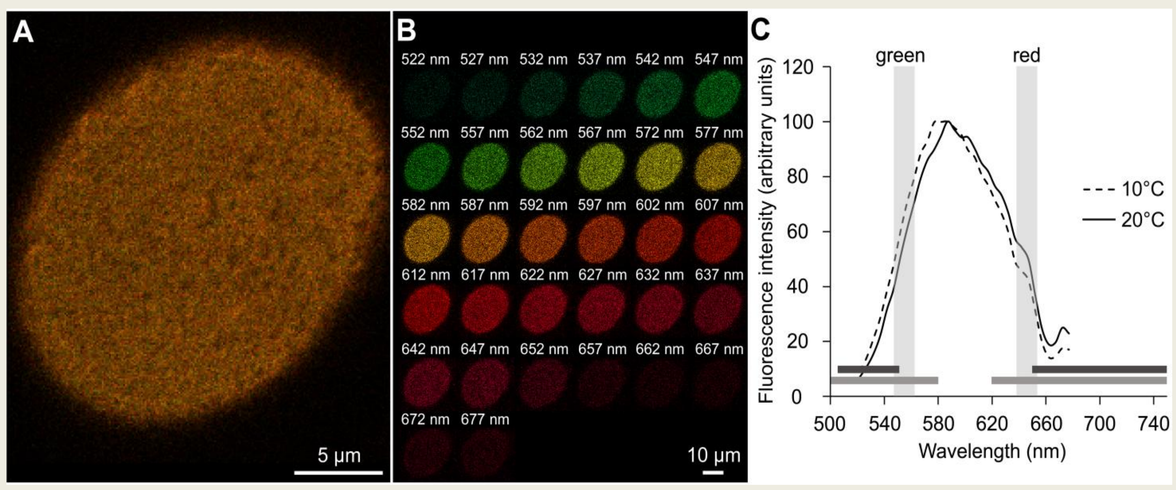
INTRODUCTION

Lipid self-association induces a physical segregation in wholly lipid bilayers, wherein a liquid-ordered Lo phase coexists with a liquid-disordered Ld phase. In the cell biology field, this preferential association of some lipids in complex mixtures has resulted in the "lipid raft" hypothesis, which proposes the formation of small (20 - 200 nm), short-lived, sterol and sphingolipid-enriched liquid-ordered assemblies; these aggregations are believed to coalesce into larger structures, which have the potential to regulate many cellular process. The function of PM compartmentalization in the detection and transduction of environmental signals in plant cells has recently emerged. Here we report the development of microscopy approach allowing the characterization of tobacco cell membrane surface organization and analyze its modification following treatment with cryptogein, an elicitor of defense reaction.

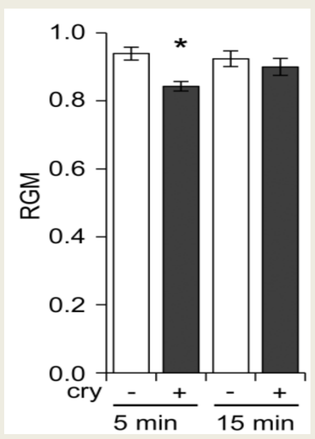
RESULTS

Characterization of PM order level in living tobacco cells using the environmental probe di-4 ANEPPDHQ.



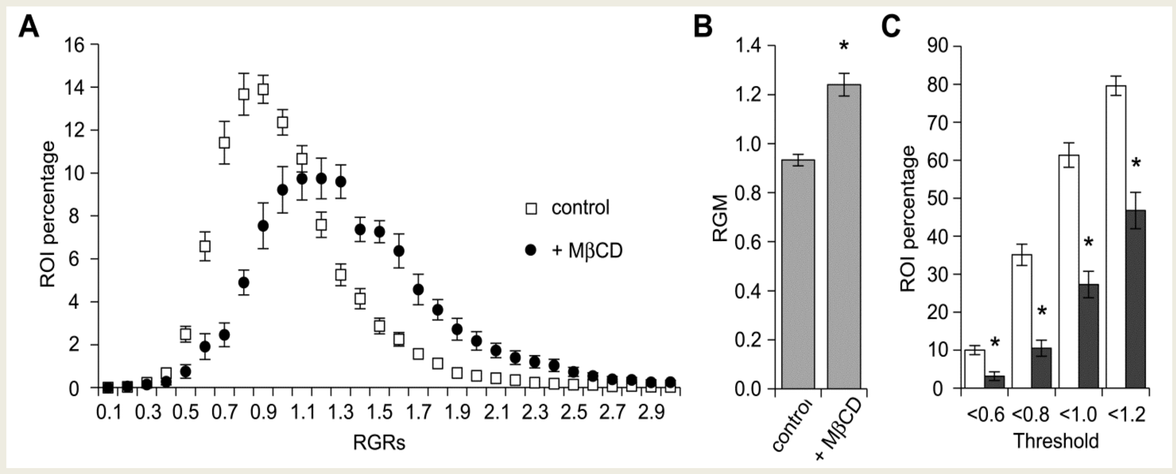
Analysis of the fluorescence emission of di-4-ANEPPDHQ is a potentially powerful approach for characterizing membrane order. After staining, tobacco suspension cells exhibit an homogeneous labeling exclusively associated to the PM (A).
Observation of a single cell was focused on the membrane surface plane allowing to acquire a large surface area. Emission fluorescence intensity was recorded simultaneously at different wavelengths using 32 independent photodetectors (B), allowing to precisely draw the di-4-ANEPPDHQ emission spectrum (C).
The order level of the membrane revealed by di-4-ANEPPDHQ fluorescence is commonly quantified using the ratio of the emission fluorescence recovered at 660 and 550 nm (I660/I550 nm).
A red/green ratio (RGM for Red/Green ratio of the Membrane) of 0.94 ± 0.02 was measured for the PM of tobacco suspension cells.

Cryptogein elicitation induced a transient modification of PM order level.



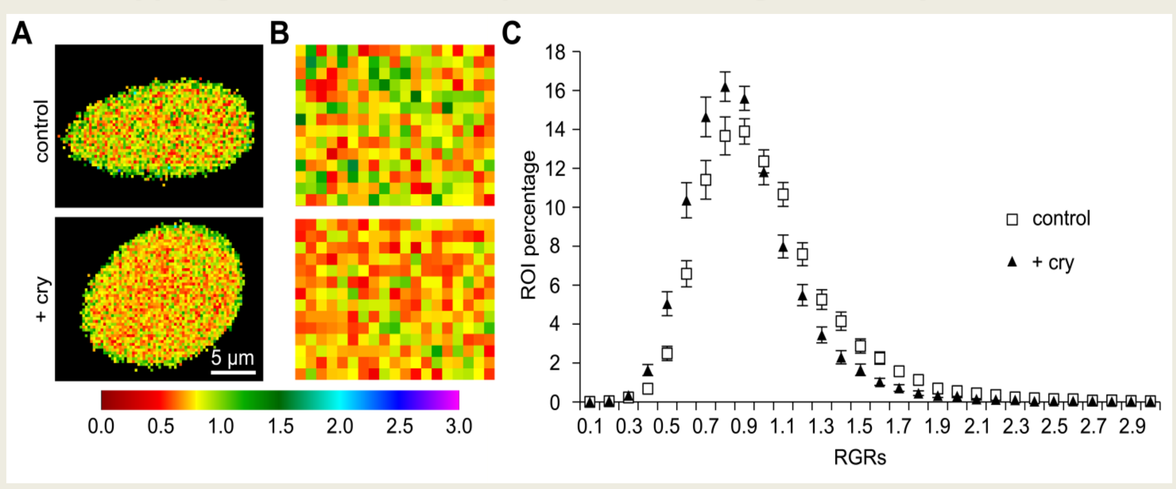
The evolution of the level of order of tobacco cell PM was followed in response to 50 nM of cryptogein.
Statistical analysis of the fluorescence of many cells at different times after treatment indicated ***a significant decrease in RGM, from 0.94 ± 0.02 to 0.84 ± 0.01, after 5 minutes of cryptogein elicitation***. Incubation with 50 nM BSA had no effect on the red/green ratio, ruling out the possibility that this modification could correspond to a non-specific effect. After 15 min of elicitation, no significant difference was observed between control- and elicited- indicating a ***transient cryptogein-induced global increase in the order level***.

Spatial organization of the tobacco cell PM order level



Dye emission spectrum was analyzed within small Region Of Interests (ROI, 300 x 300 nm). Such ROI size allows to retain sufficient fluorescence for later quantification of the red/green ratios of each ROI constituting the acquired cell surface membrane (RGR, for Red/Green ratio of the ROI).
RGR distribution was continuous, centered on 0.9, and exhibiting a wide range of values (from 0.3 to 2.5), indicating the presence of a spatial heterogeneity of the level of order of PM (A).
Treatment of tobacco cells with methyl-β-cyclodextrin (MβCD), a sterol-depleting molecule known to decrease the level of order, accordingly increased the RGM (1.3 fold, B). The distribution of the RGR was consistently shifted to higher values (A). Whatever the upper RGR threshold value used to select ROIs corresponding to the most ordered fraction of the membrane, MCD induced a significant decrease in the relative proportion of these regions (C), indicating ***a decrease in the level of tobacco PM order after sterol depletion.***

Cryptogein induced a spatial rearrangement of the tobacco cell PM order level



RGR distribution was followed after cryptogein treatment. When RGR was pseudo-color-coded, the tobacco cell PM appeared as an heterogeneous mosaic of territories of different order level (A and B). After 5 minutes of cryptogein treatment, a higher cover range of colors corresponding to lowest RGR was observed in elicited cells, confirmed by the analysis of the RGR distribution (C).
Proportions of ROIs corresponding to the most ordered fraction of the membrane was measured and a significant increase was observed after cryptogein treatment whatever RGR threshold value (0.6 to 1.2) chosen.
Cryptogein induced a significant increase of the representativeness of ROI exhibited high order level.

CONCLUSION

We characterized the sterol-dependent heterogeneity of tobacco cell PM organization and revealed the coexistence of area with a wide range of order level, opening the way to future studies, mainly based on high resolution methodologies, aiming at precising this description.
Moreover, we evidenced a modification of both global and local membrane physical properties during the early steps of a signaling process, in particular an increase in ordered domains density. These results expand our view of the function of biological membranes, while remaining within the scope of the lipid raft hypothesis. Future research endeavors will examine the underlying cellular mechanisms of this dynamic organization, which will be complemented by deciphering their precise role in the signal transduction process.