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RESÚMENES

Oil bodies dynamics and caleosin colocalization in the olive pollen during *in vitro* germination and pollen tube growth

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Oil bodies represent the primary lipid reserves in plant and animal cells. They consist of a core containing a neutral lipid (e.g. triacylglycerols, sterol esters, etc.) surrounded by a single layer of phospholipids (PLs) embedded with a few unique proteins [1]. Caleosins are oil bodies-associated proteins that contain a conserved EF-hand, calcium-binding domain [2]. Recent works suggest that these proteins might be involved in signal transduction via calcium binding or phosphorylation/dephosphorylation in processes such as membrane expansion, lipid trafficking and lipid-body biogenesis/degradation in seeds [2, 3]. Olive mature pollen grains contain large amounts of lipid bodies [4]. However, the presence and function of caleosins in pollen during germination and pollen tube growth is still a query. In this study, we have firstly monitored lipid bodies' dynamics in olive pollen during *in vitro* germination by using Nile Red staining combined with confocal laser scanning microscopy (CLSM). We observed that the number of oil bodies decreased 20-fold in the pollen grain after 6 h of germination, whereas the opposite tendency was observed in the pollen tube after 3 h of germination, indicating that oil bodies move from the pollen grain to the pollen tube. Morphometric analysis grouped lipid bodies in three classes based on size: small (0.2-0.5 µm), medium (0.5-1.2 µm) and large (> 1.2 µm) oil bodies. The number of small-sized lipid bodies quickly decreased after 1 h of germination, whereas medium and large classes increased. Then, all three classes of oil bodies diminished after 6 h of culture, but particularly the large ones. Secondly, we identified a putative caleosin of 34 kDa in the mature pollen grain after immunoprecipitation and Western blot analysis using an anti-caleosin antibody. MALDI-TOF analysis confirmed that this polypeptide corresponds to a new pollen caleosin. The expression levels of this protein decreased coincidentally with the reduction in the number of oil bodies described above. The protein was located in the periphery of oil bodies within the pollen tubes. In addition, a pool of caleosins was also visualized in the cytoplasm at the subapical zone, as well as in the vacuolar membrane at the basal zone. At later stages of germination, caleosin was preferentially located in the cytoplasm of the pollen tube and the co-localization with oil bodies was no longer observed. Our results suggest that this pollen caleosin might have a role in the mobilization of oil bodies during pollen germination and further pollen tube growth.

[1] Murphy DJ (2001*) Prog. Lipid Res*. **40**: 325.
[2] Frandsen *et al*. (2001) *Plant Physiol*. **112**: 301.
[3] Poxleitner *et al.* (2006) *Plant J*. **47**: 917.
[4] Rodríguez-García *et al*. (2003) *Protoplasma* **221**: 237.

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