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Short communication

Crystalloids in apparent autophagic plastids: Remnants of plastids or peroxisomes?

Alessio Papini [a,*], Wouter G. van Doorn [b,*]

[a] Dipartimento di Biologia Vegetale, Università di Firenze, Via La Pira 4, 50132 Florence, Italy
[b] Mann Laboratory, Department of Plant Sciences, University of California, Davis, CA 95616, USA

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ABSTRACT

Plant macroautophagy is carried out by autophagosome-type organelles. Recent evidence suggests that plastids also can carry out macroautophagy. The double membrane at the surface of plastids apparently invaginates, forming an intraplastidial space. This space contains a portion of cytoplasm that apparently becomes degraded. Here we report, in *Tillandsia* sp. and *Aechmaea* sp., the presence of almost square or diamond-shaped crystalloids inside what seems the intraplastidial space of autophagous plastids. The same type of crystalloids were observed in chloroplasts and other plastids, but were not found in the cytoplasm or the vacuole. Peroxisomes contained smaller and more irregularly shaped crystalloids compared to the ones observed in 'autophagous' plastids. It is hypothesized that plastids are able to sequester chloroplasts and other plastids.

Introduction

It has been reported that plastids seem able to sequester a portion of the cytoplasm. The outer plastid membranes apparently invaginate, forming what was called an 'intraplastidial space'. During this process a portion of the cytoplasm is taken up into the plastid, filling the intraplastidial space. Since various stages of degradation are found inside the intraplastidial space, it is possible that plastids are able to degrade parts of the cytosol and some organelles. However, the evidence that plastids can take up parts of the cytosol and organelles for subsequent degradation is mainly based on 2D TEM images of ultrathin sections. Only one first attempt has been made to use tomography for a 3D impression. These data have not yet proven that the 'intraplastidial' space becomes fully closed. Although the data suggest that plastids can function as an autophagosome and autolysosome, this idea is a hypothesis with only circumstantial support (Nagl, 1977; Gärtner and Nagl, 1980; van Doorn et al., 2011; van Doorn and Papini, 2013).

During leaf senescence the chloroplasts are dismantled. Some authors found what seemed to be remnants of chloroplasts in the vacuole (Wittenbach et al., 1982; Wada et al., 2009; Mulisch and Krupinska, 2013) but proof of dismantling in the vacuole seems as yet inadequate. Hörtensteiner (2006) was unable to corroborate chloroplast dismantling in the vacuole. Chloroplasts are at least partially degraded while still in the cytoplasm (e.g. Evans et al., 2010; Yamane et al., 2012). Compared to chloroplasts, little is as yet known about the breakdown of other plastids. The data do not exclude the possibility that chloroplasts and other plastids also can become degraded in autophagous plastids.

In yeasts and animal cells the degradation of peroxisomes is mainly or entirely due to both macroautophagy and microautophagy (Till et al., 2012). In *Ricinus communis* Vigil (1970) showed sequestering of peroxisomes (called microbodies) by plant-like autophagosomes/autolysosomes. Shibata et al. (2013) and Yoshimoto et al. (2014) corroborated autophagy of peroxisomes in plants. These data do not exclude that peroxisomes could possibly also become degraded in autophagous plastids.

Here we report the presence of crystalloids in what seems the intraplastidial space of autophagous plastids. Such crystalloids were also found in peroxisomes and plastids, but the same shape was only observed in plastids. The data suggest the hypothesis that plastids can become degraded in autophagous plastids.

Materials and methods

Flowers were collected from *Aechmaea* sp. and *Tillandsia* sp. (both genera in the Bromeliaceae) growing in the wild, between Mexico City and Cuernavaca, Mexico. Tissues studied were the central bundle cells of anthers, the pollen at the tetrad stage of

* Corresponding authors. Tel.: +31 6 10788443; fax: +31 317 413322.
E-mail addresses: alessio.papini@unifi.it (A. Papini), wgvandoorn@ucdavis.edu (W.G. van Doorn).

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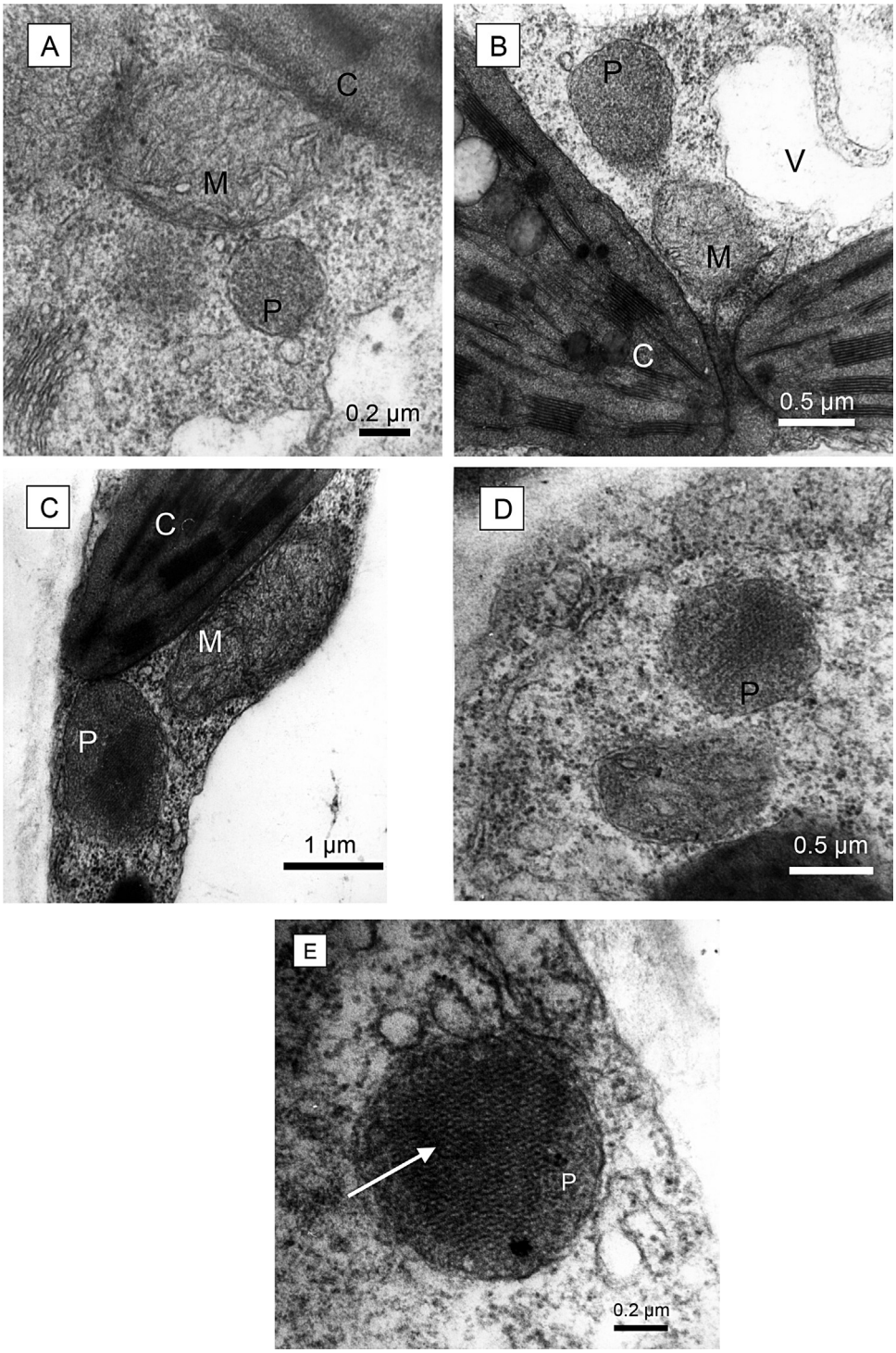

Fig. 1. Peroxisomes in central bundle cells of an anther of *Tillandsia* sp. (A) A peroxisome (the lowermost organelle) close to a mitochondrion, which is located next to a chloroplast. (B) A peroxisome (uppermost organelle at the right) close to both a chloroplast and a mitochondrion. (C) A peroxisome close to a chloroplast and a mitochondrion. The shape of a crystalloid is rather irregular. (D). Irregularly shaped crystalloid. (E) White dots of the crystalloid surrounded by electron-dense parts, similar to a honey comb (arrow). C: chloroplast; M: mitochondrion; P: peroxisome; V: vacuole. Bar sizes are indicated.

development, placenta cells in ovaries, and the parenchyma underlying the nectary as well as nectar epithelium.

Using a razor blade, transverse sections (0.5 mm thickness, about three per flower) were obtained. These sections were fixed overnight in 1.25% glutaraldehyde at 4 °C in 0.1 M phosphate buffer (pH 6.8), then post-fixed in 1% OsO_4 in the same buffer for 1 h. After dehydration in an ethanol series and a propylene oxide step, the samples were embedded in Spurr's epoxy resin. Cross sections approximately 80 nm thick were cut with a diamond knife and a Reichert-Jung ULTRACUT E ultramicrotome (about five to ten sections per thicker transverse section), stained with uranyl acetate (Gibbons and Grimstone, 1960), lead citrate (Reynolds, 1963), and then examined with a Philips EM300 TEM at 80 kV.