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Cytoplasmic channels and their association with plastids in male meiocytes of tobacco, onion and lily

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Abstract

The ultrastructures of male meiocytes in tobacco, onion and lily were studied to elucidate the interaction between cytoplasmic channels (CCs) and plastids. Before meiosis, the male sporogenous cells had identically thickened cell walls (CWs) traversed by typical plasmodesmata (PDs). After entering meiosis, their CWs became uneven in thickness and 80–500 nm aperture CCs were formed. Simultaneously, plastids or plastid-like bodies (PLBs) differing in size and morphology assembled at one or both ends of the CCs. These plastids and PLBs commonly orientated their sharper ends to face the CCs and were co-orientated on the axial line crossing the CC. Such pairs of plastids were often interconnected through the CC by thin (50–100 nm) threads emanating from their membranes. Sometimes, plastids or PLBs extended directly from one side of a CW to the other, forming a bridge via the CC. In some cases, several plastids formed bridges between cells via one common CC. This is the first report that clearly demonstrates an intercellular continuum of, or communication between, plastids in male plant meiocytes.

Keywords: Cell wall channel; Cytoplasmic channel; Intercellular plastid bridge; *Allium cepa*; *Lilium davidii*; *Nicotiana tabacum*

1. Introduction

Unlike animal cells, plant cells are encased in thick, hard cell walls (CWs) that form the plant skeleton, enabling and stabilizing three-dimensional growth. Because the CWs prevent direct contact between adjacent plant cells, various forms of cell wall channels (CWCs) have evolved to facilitate intercellular communication and co-ordinate growth and development. Recent studies have demonstrated that CWCs play a critical role, not only in plant morphogenesis (Lucas et al., 1995; Sessions et al., 2000), but also in defense responses (Voinnet et al., 1998; Palauqui and Balzergue, 1999; Mourrain et al., 2000) by controlling intercellular communication. Cytoplasmic channels (CCs) represent a type of CWC different from plasmodesmata (PD). Although CCs are plasma membrane-lined CW holes, they have a relatively large aperture compared with PD, ranging from 250 to 1750 nm (Baquar and Husain, 1969). In addition, they have no desmotubule (DT)-like core structure that exists in PD, but contains unfixed elements.

CC, also named cytoplasmic connection or cytomictic channel, was first seen in male meiocytes of *Lycopersicum esculentum* and *Cucurbita maxima* by electron microscopy (Weiling, 1965), and then in those of *Cannabis sativa* and *Dactylorchis fuchsia* (Heslop-Harrison, 1966), *Arnebia hispidissima* (Baquar and Husain, 1969), *Lilium davidii* (Zheng et al., 1987) and *Ophys lutea* (Feijo and Pais, 1988). Later they were also found in plant somatic tissues, e.g., vascular sieve plate (Esau and Thorsch, 1985), developing wheat ovules (Zhang et al., 1990), anther tapetum of *Zea mays* (Perdue et al., 1992), callus of *Nicotiana tabacum* (Guo et al., 1995) and wheat anther epidermis (Wang et al., 2004). To date, CC has been found in male meiocytes and vegetative tissues of many plant species, but because they do not occur as frequently as PD in general tissue, they have been less

Abbreviations: CC, cytoplasmic channel; CWC, cell wall channel; IPB, intercellular plastid bridge; PD, plasmadesma; PLB, plastid-like body.

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extensively studied compared with PD, so little is known of their structure and function, especially in the interaction between CC and organelles. A few previous studies have reported that plastid and mitochondria could pass through CC, but not very convincing evidence has been provided.

Recently, in the course of exploring the mechanism of CC formation we unexpectedly found that a plastid could cross a CC to form a bridge between adjacent male meiocytes of lily (Wang et al., 2002). This is a very interesting phenomenon, implying that an intercellular plastid continuum may be present in these cells. However, only one photograph was taken. To confirm the presence of the intercellular plastid bridges (IPB) or intercellular plastid continuum (IPC), a more wide-ranging investigation is necessary. This paper is just a description of our recent observations in lily and two other plants, onion and tobacco, which has demonstrated the existence of IPC.

2. Materials and methods

The plants under investigation were *N. tabacum*, *Allium cepa* and *L. davidii* var. willmottiae (wilson) Roffill. The first two species were cultivated in greenhouses, the last in the field. During the early flowering phase, whole inflorescences were excised and immersed in tap water in a flask. For electron microscope examination, the anthers in meiosis prophase I were collected, trimmed transversely into 1–2 mm long segments and immediately pre-fixed in 1.5% (w/v) paraformaldehyde (Sigma, United States) and 2.5% (v/v) glutaraldehyde in 0.025 M sodium phosphate buffer (pH 7.2) for 2–4 h at room temperature. They were post-fixed overnight in 1.5% OsO_4 (Sigma, United States) in 0.025 M sodium phosphate buffer (pH 6.8) at 4 °C, thoroughly rinsed with the phosphate buffer and dehydrated in an ethanol series. After the 100% ethanol step they were brought to room temperature and subjected to two additional changes of 100% ethanol, transferred to 1: 1 (v/v) ethanol–propylene oxide for 30 min at each step, and then to 3:1, 1:1 and 1:3 propylene–Epon 812 resin (Sigma, United States) without accelerator for 2 h for each step. Sections (70–90 nm) were prepared using an LKB-8800 ultra-thin microtome (LKB, Sweden) and stained with 2% (w/v) aqueous uranyl acetate and 2.6% (w/v) lead citrate. Finally, they were observed under a Philips EM-400T transmission electron microscope (TEM) (Phillips, The Netherlands) set at 80 kV.

3. Results

The male sporogenous cells of all three plants examined shared general meristematic features before meiosis: smaller volumes with large, dense nuclei. Their CWs were of constant thickness and traversed by typical PDs, marked by a single 30-nm thin and highly electron-opaque thread. During early prophase (leptotene to early pachytene stage) of meiosis I, the cells became larger and rounder in volume and dimension. Their nuclei were centrally located and cytoplasm contained more plastids, vacuoles and ERs. The plastids were sphere-, oval-, tadpole-, line- or dumbbell-shaped, with stroma containing tubular structures and starch, showing more polymorphism than the sporogenous phase. Their CWs were thickened, and consisted of clear middle layer (ML), primary wall (PM) and irregularly thickened callose layer. On the CWs, the PDs observed at earlier phase were rarely observed, but CCs ranging from 80 to 500 nm in aperture were formed. In some cases, CCs were found containing cytoplasmic matrix, ribosome and occasionally nuclear material, within and near which no plastid existed (data not shown). However, in other cases, they were apparently associated or connected with plastids in the following patterns.

(1) There were several plastids at each end of a CC, each appearing to be paired with another at the opposite end, and directing its sharper apex towards the CC. The paired plastids were orientated on the same axial line across the CC and a thin thread connected one to the other via the CC, thereby resulting in a bridge between neighboring cells. The linking thread appeared highly electron dense and emitted from the plastid. The CCs were 150–250 nm in aperture, apparently wider than the thread (Fig. 1A–C). In addition, it was found that several plastids assembled at one end of a CC and directed their sharper tops into the CC, exhibiting a tendency to pass through the CC (Fig. 1D).
(2) There was one plastid at each end of a CC. Both plastids lying at different sides of CW co-orientated on the same axial line across the CC and directed their sharper apex toward the CC. A highly electron-opaque thread apparently emitting from one plastid extended to the opposite of CW and connected to the plastid there. The CC was curved (Fig. 2A) or straight (Fig. 2B), and the threads were as wide as the CC's aperture.
(3) A single long intact plastid directly extended from one side to the other side of CW to form a bridge between adjacent cells via a CC (Fig. 2C and D). The CCs were 150–500 nm in aperture and the bridge, the portion embedded in the CCs, was as wide as the CC's aperture (Fig. 2C and D) or thinner (Fig. 3A and D) than it.
(4) There was one plastid at each side of CW. The two plastids co-orientated on the same axial line across and perpendicular to the CW, and embedded their sharper ends into CW. However, a complete CC was invisible at CW between the plastids, perhaps due to inappropriate CC plane (Fig. 3B and C). The two plastids may be equivalent to two halves of one plastid.
(5) Some plastid-like bodies, marked by very highly electron-opaque stroma free of both starch grains and membranous tubules were observed to expand through the CC to form a bridge between adjacent cells (Fig. 3D and E) or to lie within a CC (Fig. 3F).

4. Discussion

4.1. Features of the intercellular plastid bridge

The above results clearly demonstrate the presence of a close association or interaction between CC and plastid in the three plants examined, which was characterized by the formation of intercellular plastid bridges (IPBs). The IPBs were of two types: one was marked by direct extension of a plastid from one side of the CW to the other via the CC, and the other by a thin thread that was emitted from a plastid and linked to another plastid on the opposite side of CW via a CC.