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BRIEF COMMUNICATION

Plastid-nuclear complexes in the photosynthesizing cells from their mitosis up to programmed death

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Abstract

Permanent plastid-nuclear complexes (PNCs) exist in tobacco cells from their mitosis up to programmed cell death (PCD). PNCs in senescing cells of tobacco leaves were typical by enclosure of peroxisomes and mitochondria among chloroplasts which were in contact with nucleus. Such a complex position provides simultaneous interaction of these organelles and direct regulation of metabolism and PCD avoiding the cytosol.

Additional key words: chloroplasts; *Nicotiana tabaccum*; nuclei; programmed cell death.

Despite the long research of chloroplasts, the notion that they are localized randomly in a cell is still popular. The location of chloroplasts around the nucleus was demonstrated in *Euglena gracilis* (Ehara *et al.* 1985), during action of a retardant (Selga and Selga 1995), and in relation to plastid stromules (Kwok and Hanson 2004). Regular presence of permanent PNCs in photosynthesizing tissues of vascular plants, recently demonstrated by Selga *et al.* (2010), provided the new perspective to examine the mechanisms, which control both developmental and death processes in plants. In this work, participation of PNCs in the mitosis, life span regulation, and PCD of the tobacco leaf cells was presented.

Tobacco (*Nicotiana tabaccum* L.) plants were cultivated in soil culture, under natural light, and day/night temperatures of 19–25°C. They were decapitated to develop a source-sink system, where promoted side shoots accelerated PCD in the near located leaves of the main stalks.

Bright field microscopy: Pieces of mesophyll from the small (1–1.5 cm long) leaves of promoted side shoots were treated with methanol-acetic acid (3:1), rinsed in distilled water, macerated in 1 M HCl at 60°C, and contrasted with acetic orceine.

Epidermal peels of yellowing leaves of the main stalks were fixed with acetic ethanol and contrasted with acetic orceine. Samples were examined under a light microscope *Leica DM 2000* (*Leica Microsystems*, Germany). Photographs were taken with a digital camera *Leica EC 3* (*Leica Microsystems*, Germany) and images processed with *LAS EZ* and *Paint Shop Pro 4*.

Laser confocal microscopy: Mesophyll from the small leaves of promoted side shoots and epidermal peels were analyzed using a *Leica DM RA-2* microscope (*Leica Microsystems*, Germany) equipment with a *TCS-SL* confocal scanning head (*Leica Microsystems*, Bannockburn, USA). Images were collected with a *Leica 40 X HCX PL Fluator* objective (NA = 0.75) and 100x *HCX PIAPO* oil immersion objective (NA = 1.40). Green fluorescent protein (GFP) was excited with a 488 nm band from four-line argon ion laser. Chlorophyll fluorescence was excited at 633 nm. GFP fluorescence was detected between 650–715 nm. Propidium iodide fluorescence was detected between 600–620 nm, 4',6-diamidino-2-phenylindole (DAPI) at 480 nm.

Transmission electron microscopy: Pieces of mesophyll from the senescing leaves were fixed repeatedly both with 2% (v/v) glutaraldehyde and 4% (v/v) osmium tetroxide in 0.2 M sodium cacodylate buffer, dehydrated and contrasted with 1% phosphowolframic acid and embedded in a mixture of epoxy resin (*Epons 812*, *MNA*, *HY 946*, *DYO 64; Fluka AG*, Switzerland). Ultrathin sections were cut with the ultra microtome *LKB8800* (*LKB*, Sweeden), contrasted with lead citrate and examined with

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Abbreviations: GFP – green fluorescent protein; PCD – programmed cell death; PNC– plastid-nuclear complexes.
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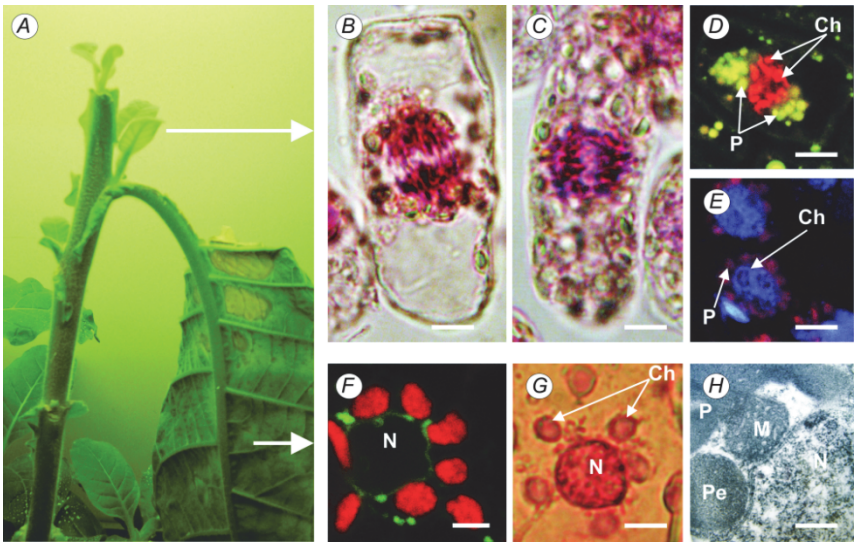

Fig. 1. Plastid-nuclear complexes (PNCs) in dividing and senescent cells of tobacco (*Nicotiana tabaccum L.*). *A*: the tobacco plant a week after decapitation; *B,C*: chloroplasts encircles area of separating choromosomes during mitosis of palisade parenchyma cells in small sink leaves, fixed with methanol-acetic acid (3:1) and stained with acetic orceine, scale bars = 5 μm; *D*: chloroplasts in close proximity to dividing chromosmes during mitosis, fixed with paraformaldehyde and stained with propidium iodide, chlorophyll autofluorescence - green, propidium iodide - red, scale bar = 5 μm; *E*: chloroplasts in close proximity to dividing chromosmes during mitosis *in vivo*, chlorophyll autofluorescence - red, DAPI - blue, scale bar = 5 μm; *F*: daisy-shaped PNC of epidermal cell *in vivo* during senescence, confocal laser scanning microscopy, autofluorescence of chlorophyll (red) and GFP fluorescence (green) in nuclear envelope, endoplasmic reticulum and Golgi bodies, scale bar = 3 μm; *G*: a cytoplasmic bridge connecting PNC with plasma membrane and plasmodesmata; *H*: a direct contact among outer envelope membranes of nucleus, chloroplast, mitochondria, peroxisomes, transmission electron microscopy, scale bar = 300 nm. P – plastid, Ch – chromosomes, N – nucleus, M – mitochondrion, Pe – peroxisome.

transmission electron microscopes *TESLA BS 500* (*TESLA*, CR) under magnification of 2,000 to 25,000.

Decapitation of tobacco plants caused promotion of several auxiliary buds and accelerated PCD in the adjacent leaves of main stalks (Fig. 1*A*). The small leaves of developing side shoots showed a well differentiated and mitotically active palisade and spongy mesophyll. In all types of cells, the population of plastids could be divided in two categories – those that were permanently joined in PNC and the rest of randomly scattered plastids. The palisade cells divided both by periclinal and anticlinal divisions (Fig. 1*B*,*C*). PNCs participated in a spatial regulation of mitosis: some plastids encircled mitotic spindle and formed more or less separated compartment from the rest of a cell. Moreover, some plastids of PNC retained a close proximity to the mitotic spindle and chromosomes (Fig. 1*D,E*). Epidermal cells of yellowing leaves of the main stalks contained particularly pronounced daisy-shaped PNCs. A part of the scattered chloroplasts was smaller with weaker chlorophyll autofluorescence. The number of plastids involved in PNCs and closeness of their structural interaction with nuclei was variable: direct mutual contact of membranes over a wide surface area (Fig. 1*F*), or joining plastids reciprocally to nucleus and to plasmalemma by longish connective structures (Fig. 1*G*). In addition, regular enclosure of peroxisomes and mitochondria in the PNCs by joining their envelope membranes in wide areas (Fig. 1*H*) provided simultaneous and direct feedback interaction of these organelles and regulation of the PCD process.

The maintenance of mitotic activity in the differentiated cells of growing leaves is well known. However, the relationships between mitotic mechanisms and cell differentiation are not sufficiently investigated (Bannigan *et al.* 2008). Unlike the popular notion of open mitosis that occurs in undifferentiated stem cells of plant root and shoot apical meristems (Singh and Bhalla 2006), the present results suggested that differentiated palisade cells retained the mitotic activity, but the presence of PNC switched the mechanism of mitosis to a partly closed ancient manner as far as endoplasmic reticulum and nuclear envelope attached to plastids moved away from chromosomes, but they did not break completely. Induction of plant PCD and the role of plastids in this process has been widely discussed (Doorn *et al.* 2011), taking into account reports about attachment of chloroplasts to nucleus (Possingham 1980), participation of plastids in initiation of PCD (Samuilov *et al.* 2002, Zapata *et al.* 2005), and about direct plastid to nucleus signalling (Pogson *et al.* 2008, Bräutingam *et al.* 2009). The present results suggest that permanent PNCs containing also mitochondria and peroxisomes exist up to the very end of PCD and that the destruction of chloroplasts joined with nuclei occurs later and probably in a different way in comparison with the scattered chloroplasts.

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