

SHORT COMMUNICATION

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## Morphological and quantitative changes in mitochondria, plastids, and peroxisomes during the log-to-stationary transition of the growth phase in cultured tobacco BY-2 cells

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### ABSTRACT

We developed a wide-range and high-resolution transmission electron microscope acquisition system and obtained giga-pixel images of tobacco BY-2 cells during the log and stationary phases of cell growth. We demonstrated that the distribution and ultrastructure of compartments involved in membrane traffic (i.e., Golgi apparatus, multivesicular body, and vesicle cluster) change during the log-to-stationary transition. Mitochondria, peroxisomes, and plastids were also enumerated. Electron densities of mitochondria and peroxisomes were altered during the growth-phase shift, while their numbers were reduced by nearly half. Plastid structure dramatically changed from atypical to spherical with starch granules. Nearly the same number of plastids was observed in both log and stationary phases. These results indicate that mechanisms regulating organelle populations differ from organelle to organelle.

**Abbreviations:** BY-2, Bright yellow 2; EM, electron microscopy; TEM, transmission electron microscopy

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### KEYWORDS

High-pressure freezing; mitochondrion; peroxisome; plastid; transmission electron microscopy; tobacco BY-2 cells


Undifferentiated or partially differentiated plant cells can change their shape in response to the environment. As the shape of the cells changes, organelles change their function using limited components. However, the mechanism by which organelles differentiate is poorly understood, as is the method by which differentiation is defined by alterations in changes in structure and function. Morphological changes at different growth phases can be clearly observed in cells cultured in suspension, such as the in the logarithmic growth phase and the stationary phase; however, changes in organelle differentiation and distribution during the different phases are not well understood. The movement and distribution of organelles can be visualized using fluorescence microscopy techniques, although these techniques do not permit the observation of the ultra-structure of small organelles. For example, the shape of large vacuoles, the nucleus, and the cell itself are easily detected using a light microscope, but other organelles are difficult to discern in detail, and can only be seen as dots using a fluorescent microscope. Recently, we observed tobacco BY-2 cells prepared by high-pressure freezing method using a wide-range and high-resolution transmission electron microscope (TEM) acquisition system developed in our lab. We have previously reported that the number of vesicle clusters in tobacco BY-2 cells decreased and the Golgi stack thinned down during the transition from the log to the stationary phase, whereas neither the number nor the shape of the multivesicular bodies changed during either stage.<sup>1</sup> Over the course of this study,

we also observed changes in mitochondria, peroxisomes, and plastids, and report these changes here.

Mitochondria were spherical and approximately 800 nm in diameter during the log and stationary phases (Fig. 1B and G). Most mitochondria in the log phase had a low electron density matrix, whereas mitochondria in the stationary phase showed a high-density matrix. Occasionally, mitochondrial fission was observed during the log phase. The number of mitochondria ranged from 19.2 to 28.1 per 100  $\mu\text{m}^2$  of cytoplasmic region in the log phase and from 10.6 to 22.4 per 100  $\mu\text{m}^2$  of cytoplasmic region during the stationary phase (Table 1), while the average number of mitochondria in cells was reduced by nearly half during the transition from the log to the stationary phase (Table 1 and Fig. 2A).

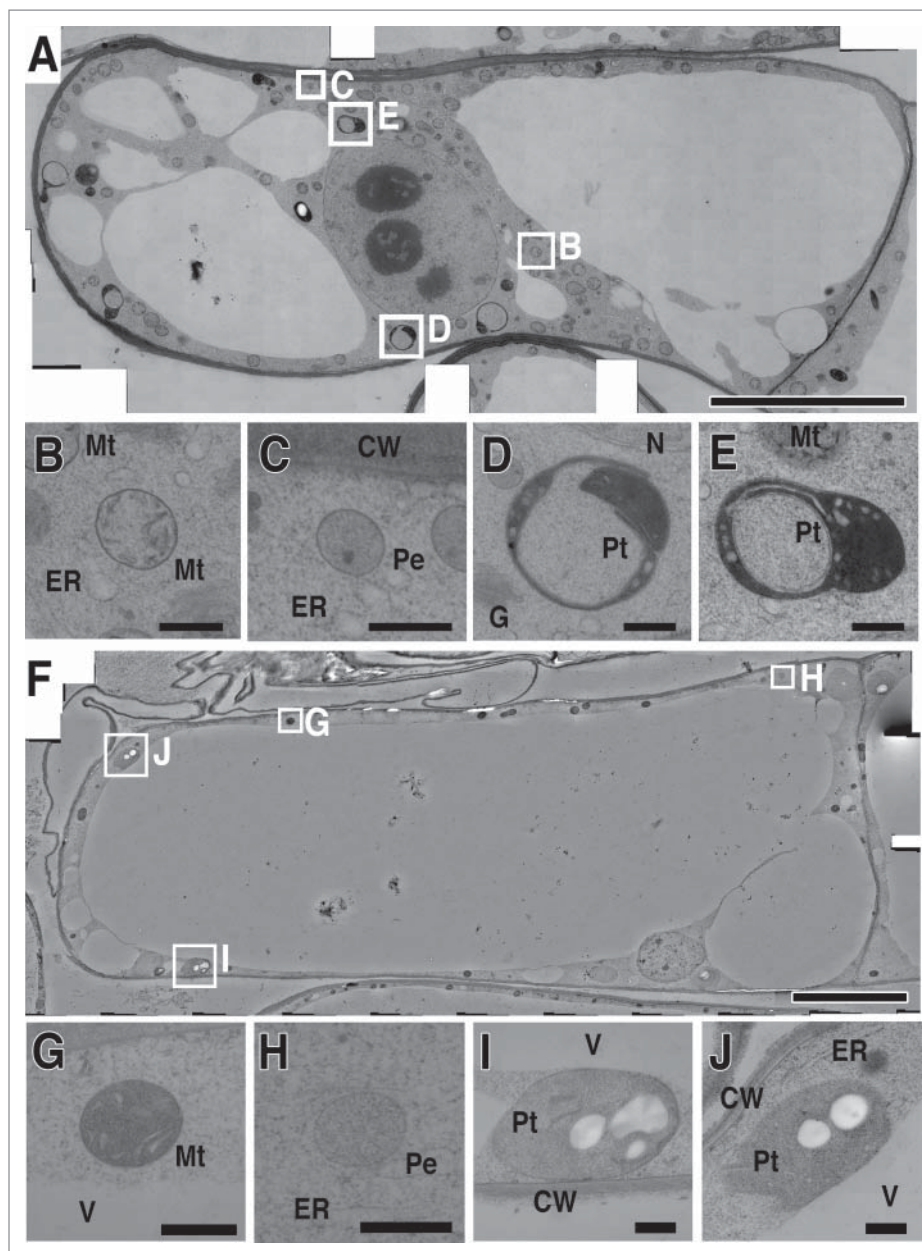
Peroxisomes were round, with an average diameter of 392 nm in the log and 455 nm in the stationary phase (Fig. 1C and H). Several peroxisomes in the log phase contained a high-density core; however, this structure was not observed during the stationary phase (Fig. 1C and H). The density of peroxisome content during the stationary phase was lower than that of the log phase. The number of peroxisomes ranged from 4.9 to 5.2 per 100  $\mu\text{m}^2$  in the log phase and from 2.0 to 3.6 per 100  $\mu\text{m}^2$  in the stationary phase (Table 1). Similarly to mitochondria, the average number of peroxisomes in a cell was reduced by nearly half during the transition from the log to the stationary phase (Table 1 and Fig. 2B).

Plastids in the log phase had tubular sheet-like structures with more electron-dense stromal regions (Fig. 1A, D, and E).

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**Figure 1.** Ultrastructure of mitochondria, peroxisomes, and plastids in a high-pressure frozen BY-2 cell during the log and stationary phases. A cell in an ultra-thin section was captured by an auto-acquisition transmission electron microscopy (TEM) system. White squares in the image of the whole cell section in the log phase (A) correspond to magnified images of the mitochondrion (Mt) (B), peroxisome (Pe) (C), and plastid (Pt) (D and E). White squares in the image of the whole cell section in the stationary phase (F) correspond to the magnified images of the mitochondrion (Mt) (G), peroxisome (Pe) (H), and plastid (Pt) (I and J). ER, endoplasmic reticulum; V, vacuole; CW, cell wall. Bars = 10  $\mu\text{m}$  in (A) and (F); 500 nm in (B)–(E) and (G)–(J).

A few plastids contained small starch granules (Fig. 1A). The plastids in stationary-phase cells had lower electron densities in stromal regions and were larger and more elliptical than those

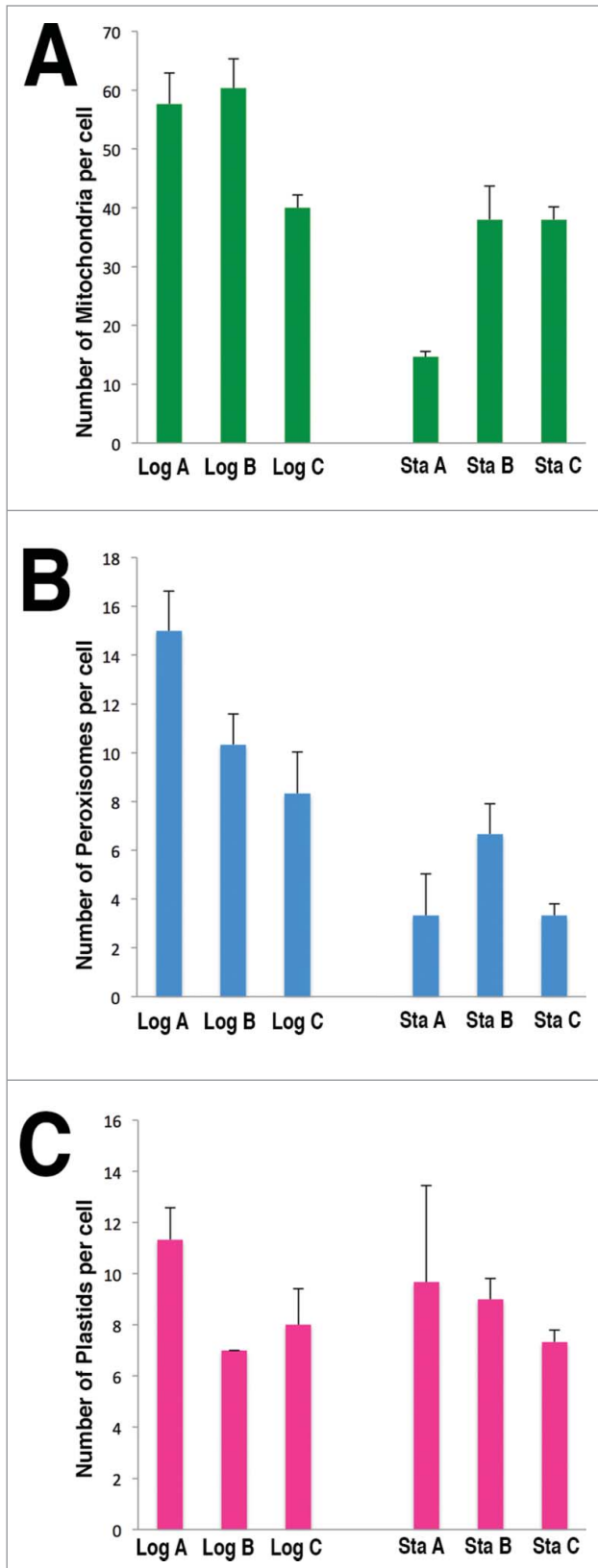
in log-phase cells (Fig. 1A, D, E, F, I, and J). The starch granules in plastids during the stationary phase were relatively large (Fig. 1I and J). Interestingly, the number of plastids in the cells was 4–7 per section and the organelle density was 4.3–7.0 per 100  $\mu\text{m}^2$  in the cytosolic region of stationary-phase cells, nearly identical in density to the log-phase cells (3.3–4.8 per 100  $\mu\text{m}^2$ ; Table 1 and Fig. 2C).

**Table 1.** Numbers of mitochondria, peroxisomes, and plastids in 100  $\mu\text{m}^2$  of cytoplasmic region in three cells during the log and stationary (Sta) phases. The cytoplasmic region was defined as the total area inside the plasma membrane, excluding the areas of vacuoles and nuclei. Values represent organelle number per 100  $\mu\text{m}^2$ .

	LogA	LogB	LogC	StaA	StaB	StaC
Mitochondria	28.1	19.2	23.2	10.6	2.4	21.3
Peroxisomes	4.9	5.2	5.0	2.4	3.6	2.0
Plastids	3.3	4.0	4.8	7.0	4.8	4.3

### Conclusion and perspective

The ultrastructure and density of organelles varied in tobacco BY-2 cells during the log and stationary phases. This suggests the possibility that osmiophilic constituents are dramatically altered in organelles by the regulation of organelle-specific



**Figure 2.** Total number of mitochondria, peroxisomes, and plastids in longitudinal sections of whole cells during the log and stationary phases. Mitochondria, peroxisomes, and plastids were counted in three cells during the log and stationary (Sta) phases. Graphs show the number of (A) mitochondria, (B) peroxisomes, and (C) plastids per cell from log and stationary phases. Error bars represent the SE of counts obtained by three individuals.

protein expression during the log-to-stationary transition. Here, we reported that the number of peroxisomes and mitochondria were reduced almost by half in cells in the stationary phase (Table 1 and Fig. 2A and B). It is known that regulatory systems for organelle populations include fission and fusion,<sup>2,3</sup> degradation by autophagy pathways,<sup>4</sup> and other mechanisms.<sup>5</sup> Previously, we reported that protein aggregates in tobacco BY-2 cells are transported to vacuoles by autophagy.<sup>6</sup> Recent studies have shown that peroxisomes and mitochondria are degraded by selective autophagy in tobacco BY-2 cells<sup>7</sup> and *Arabidopsis*,<sup>8</sup> which is controlled by nutrient limitation.

In our study, however, the number of plastids remained nearly unchanged during the phase transition. A previous study that used fluorescent microscopy reported that the number of plastids per BY-2 cell was hardly changed at 3 and 7 days after transfer to a new medium.<sup>9</sup> Interestingly, we found that the electron density of the stroma in plastids in the stationary phase was lower than that of stroma in the log phase according to our observations. It was reported that stroma-targeting green fluorescent protein was transferred to the vacuoles in wheat, rice, and *Arabidopsis* through the rubisco-containing body (RCB) pathway.<sup>10,11</sup> Given that the ultrastructure of plastids differed considerably between the log and stationary phases in our study, although numbers were not significantly affected, plastid contents might be degraded via RCB during the transition between cell phases. Plastids in cells during the log phase have a typical shapes (i.e., tubular sheet-like structures), changing into round or elliptical structures in the stationary phase. This observation suggests that both fission and the reduction of metabolic activities affect the morphology of plastids. Recently, quantitative electron microscopic observations of plastids from the embryogenic microspores of *Brassica napus* revealed that approximately 60% of plastids were round or bean-like in shape, while other plastids presented atypical shapes similar to those observed during the log phase in the present study, and that these dramatic structural changes are related to a cytoplasmic cleaning mechanism.<sup>12</sup> The dynamic morphological changes in plastids in BY-2 cells may play an important role in the degradation of other cellular components if a similar system is present in tobacco. Moreover, a relatively large number of plastids contained large starch granules during the stationary phase (Fig. 1A, D, E, F, I, and J), possibly owing to an imbalance of auxin and cytokinins.<sup>13</sup>

We documented the changes in the ultrastructure and distribution of organelles in BY-2 cells between the log and stationary phase using a wide-range and high-resolution TEM acquisition system. However, it is difficult to obtain ultra-thin longitudinal sections of tobacco BY-2 cells, especially from cells in the stationary phase. Moreover, the number of organelles varies in cells in the same growth phase, which could possibly be the result of individual differences among the cells or to the random distribution of organelles within each cell. We acknowledge the limitations of our present data, owing to the use of two-dimensional EM images from sections that were only 80 nm thick. To measure the correct ultrastructural and distributional data, novel three-dimensional EM techniques would be required in the future. Moreover, investigating the ultrastructure and distribution of organelles in the tissues and organs of plants under various environmental conditions would

further elucidate how cell differentiation affects intracellular structures.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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