

Discovery of Three Rubber Particle Ontogeny Pathways during *Taraxacum kok-saghyz* Rubber Formation

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## ABSTRACT

Information on rubber particle ontogeny and its *de novo* site is very limited in *Taraxacum kok-saghyz* (TK), an alternative temperate zone rubber crop suited to cultivation in the US and Europe. Microscopic analyses of plants from seedlings to 1-yr-olds, as well as plants grown in cold temperatures, shows that there are three different pathways for rubber particle ontogeny in root laticifer cells. Our study indicates that rubber particles originate in the endoplasmic reticulum (ER)-Golgi apparatus vesicular complex. Under ambient temperatures, rubber particles were produced in two distinct ways. Some particles were produced by laticifer plastids, membranous organelles found in the cytoplasm of laticifer cells (plastidic rubber). Other non-plastidic particles (vesicular rubber) were often located near or in the tonoplast. In contrast, rubber particles from cold-grown roots appear to be produced from compressed layers of endoplasmic reticulum located very close to thick cell walls, without participation of the Golgi apparatus. Nevertheless, rubber particle development after ontogeny was similar to vesicular rubber particle development. The cold-grown roots also contained higher rubber content after 50 days than ambient-grown roots. For the first time, an understanding of rubber particle ontogeny in TK and its development under abiotic cold treatment has been obtained. This information may inform crop management to enhance crop yield.

## INTRODUCTION

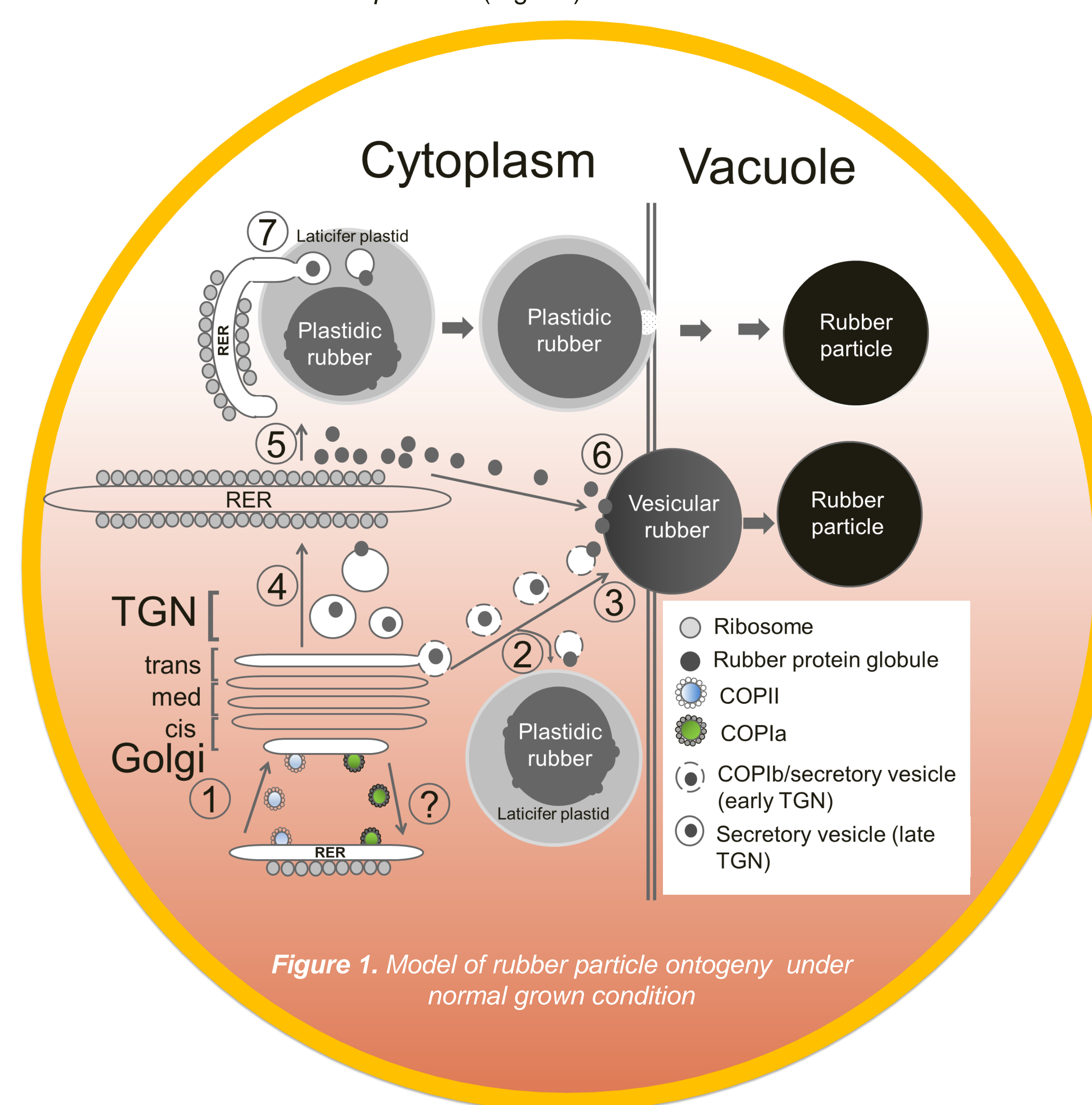
*Taraxacum kok-saghyz* (TK), or rubber dandelion, is in the Astereacea family and native to Central Asia. It contains pipe-like multi-nucleate vessels, mostly in the roots, called laticifers that produce high-quality rubber and it can be grown in temperate climates [1]. These characteristics have made this species a potential source for natural rubber production. Histological and ultrastructural studies of rubber storage vessels in different species, such as *Hevea brasiliensis* (rubber tree), the world's main rubber producing tropical plant, and *Parthenium argentatum* (guayule), an alternative rubber crop for semi-arid regions, suggest sites of *de novo* rubber formation. However, no information is available for TK. Thus, the objective of this study was to examine rubber ontogeny in TK using transmission electron microscopy, with the intention of identifying the subcellular site of rubber particle origination, and to follow the movement of rubber particles through vesicular trafficking cellular pathways. Another objective is to microscopically characterize cold-induced changes in the ontogeny of rubber particles to understand the dynamics of rubber particle ontogeny under stressful conditions.

## MATERIALS AND METHODS

TK plants of mixed phenotypes were established and grown in outside planting boxes under normal grown conditions. Plants were harvested for histology samples every 2 months for 1 year. For cold stress studies, the plants are left to grow, reaching 6 and 12 months old in November 2016. The boxes were either left uncovered to experience snow or were covered with plastic. Plants were harvested after 25 and 50 days (latex content was quantified) [2]. All root samples were fixed, dehydrated, and resin-infiltrated before being sectioned, stained, and viewed under a transmission electron microscope (TEM) (Hitachi H-7500).

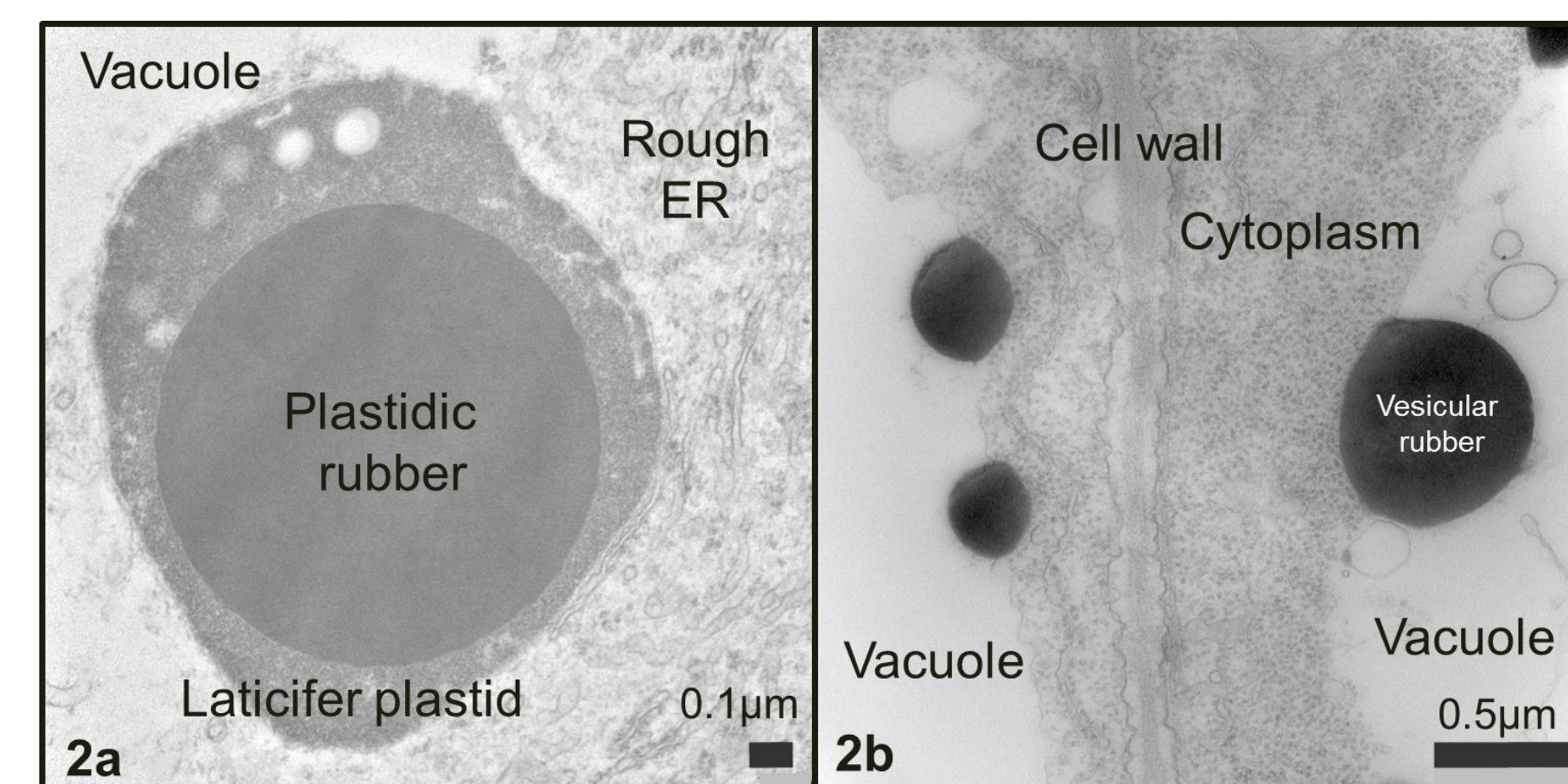
## RESULTS AND DISCUSSION

Two distinct rubber particle morphologies and ontological localities were characterized in TK roots. Laticifer plastids, membranous organelles found in the cytoplasm of laticifer cells, produced some of the rubber particles, termed *plastidic rubber particles* (Fig. 2a). Other rubber particles were formed independently of these plastids, often at the tonoplast, and were termed *vesicular rubber particles* (Fig. 2b).

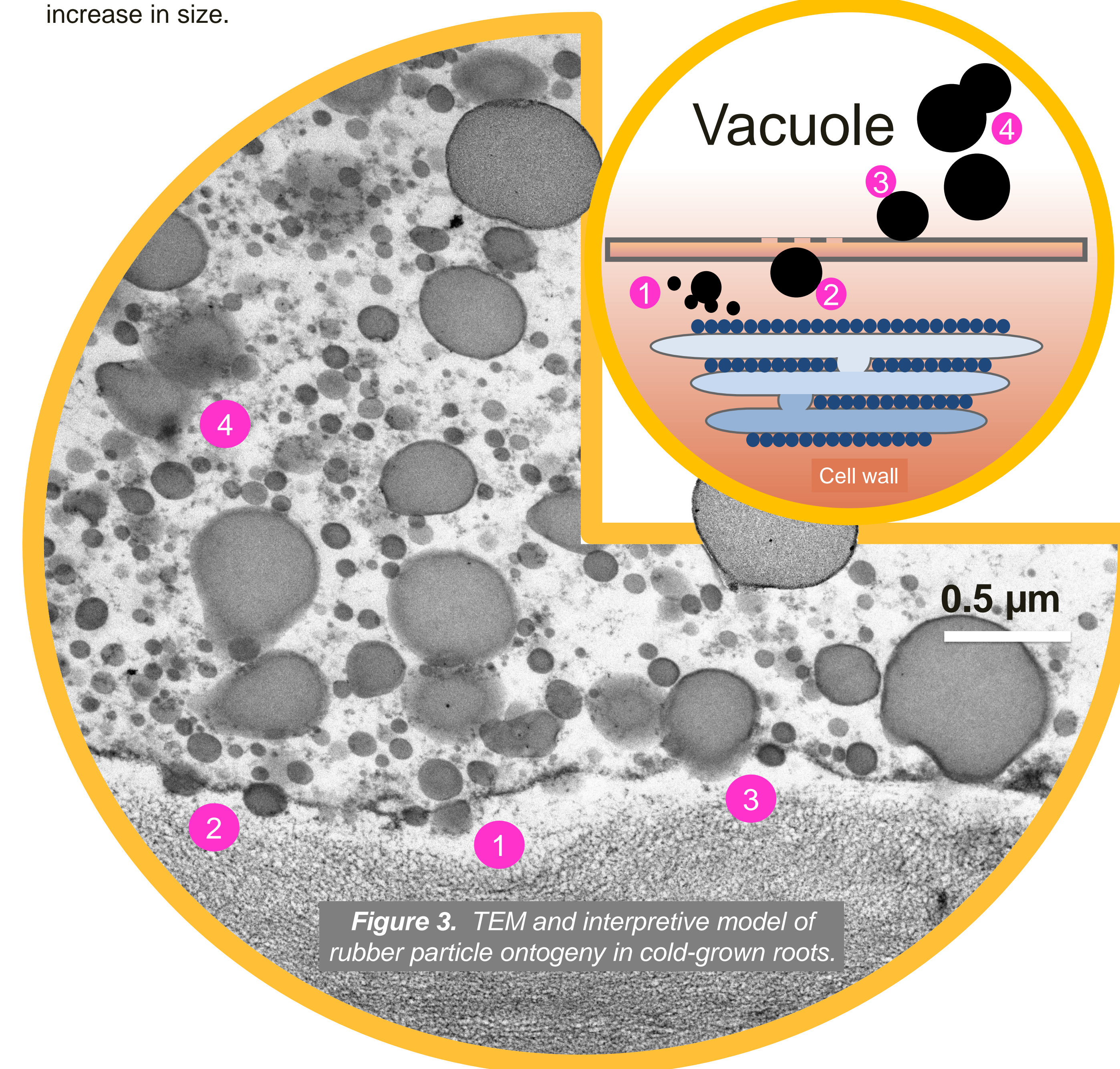


Both forms of rubber particle formation involved the endoplasmic reticulum (ER)-Golgi apparatus vesicular complex.

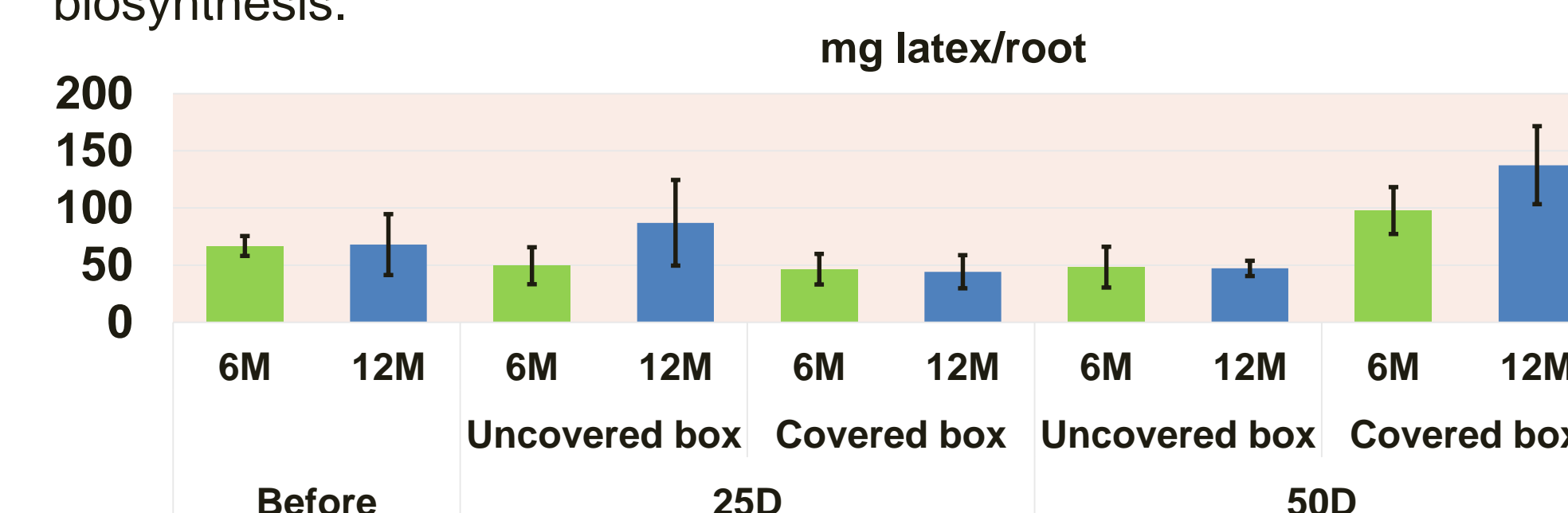
- ① The coat protein complex II (COPII) route or the anterograde pathway.
- ② The COPIa (the retrograde pathway from the Golgi to the ER) could not be observed. The budding of COPIb and the production of secretory vesicles from *medial* and *trans*-Golgi area release rubber globules either into: ② laticifer plastids rubber or ③ directly to form vesicular rubber particles.
- ④ Secretory vesicles containing rubber globules form in the late *trans*-Golgi network (TGN) and *medial* area of the Golgi, and are transported back to the rough ER (RER). As they leave the RER, the rubber globules will either: ⑤ move into laticifer plastids to form plastidic rubber or ⑥ directly form vesicular rubber.
- ⑦ Lastly, the RER connects directly to the laticifer plastid and transfers the rubber globules into the plastid to form plastidic rubber.



Another rubber particle ontogeny pathway was discovered in cold-grown roots. These rubber particles appear to be produced from compressed layers of endoplasmic reticulum, located very close to thick cell walls, without participation of Golgi apparatus as occurs in ambient temperatures (Fig.3). However, rubber particle development is similar to vesicular rubber particle production. It begins with (1) the accumulation of rubber globules to form a rubber particle; (2) the rubber particle moves near the tonoplast; (3) the rubber particle is ejected into the vacuole; (4) in the vacuole, the rubber particles coalesce with other rubber particles and increase in size.



The cold-grown roots also contained higher rubber content after 50 days than ambient-grown roots. Higher latex contents were observed in roots from covered boxes than uncovered boxes after 50 days (Fig. 4), suggesting that colder temperatures and snow cover inhibited rubber biosynthesis.



**Figure 4. Root latex content in plants grown in outdoor boxes for 25 and 50 days (means of 12 ± se).**

## CONCLUSIONS

In this study, we established the first *in vivo* model for rubber production in TK. The model supported the view that rubber particles begin formation in the endoplasmic reticulum (ER)-Golgi apparatus vesicular complex. Rubber particle ontogeny in the cold differs from particle formation at ambient temperatures, as it appears Golgi bodies are not involved.

## REFERENCES

- [1] Hodgson-Kratky, K.J.M., Wolyn, D.J. (2015) J. Amer. Hort. Sci. 140: 614-619
- [2] Pearson, C.H. *et al.* (2013) Ind. Crops and Prod. 43: 506-510

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