The Plant TGN, a Challenge for Light- and Electron Microscopy

York-Dieter Stierhof[1], Elizabeth F. Crowell[2], Karin Schumacher[3]

1. Center for Plant Molecular Biology, Microscopy, University of Tübingen, 72076 Tübingen, Germany
2. Laboratoire de Biologie Cellulaire, Institut National de la Recherche Agronomique, 78026 Versailles cedex, France
3. Heidelberg Institute for Plant Sciences, University of Heidelberg, 69120 Heidelberg, Germany

york.stierhof@zmbp.uni-tuebingen,de
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Early endocytic and secretory trafficking converge in the plant trans-Golgi network (TGN) [1,2]. Owing to the small size of the TGN/early endosome (EE) and the mobility of plant Golgi stacks, it is challenging to identify subdomains of this multifunctional compartment. In addition, TGN and Golgi associated transport vesicles like CCV, COPI and COPII vesicles are difficult to visualize in resin embedded samples as well as in thawed cryosections used for immunogold labelling, probably due to their small size [3]. A number of antigens, either components or cargo of the endocytic or secretory trafficking machinery have be localized to the plant TGN/EE but it remains to be determined if they can be used to identify functional subdomains. Moreover, the specific contribution of TGN-derived vesicles and tubules to the so-called BFA compartment, a vesicle cluster induced by the fungal macrocyclic lactone brefeldin A, is not completely clear.

We present immunolabelling data for several antigens involved in vesicle trafficking and a carbohydrate epitope. In addition, we compare different thin section labelling methods: i) Tokuyasu thawed cryosection labelling after chemical fixation or ii) after high-pressure freezing, freeze- substitution and rehydration [4,5] and iii) resin section labelling after high-pressure freezing and freeze-substitution. We also demonstrate that the TGN shows a surprising mobility.

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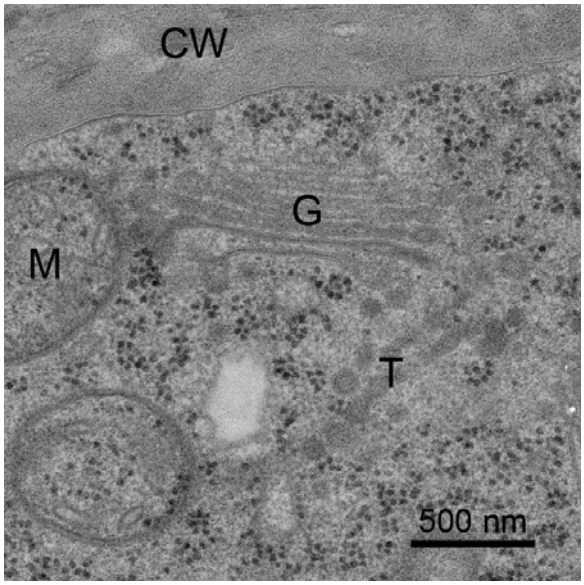

Figure 1. Ultrathin resin section of a Golgi stack and associated TGN after high-pressure freezing, freeze-substitution and resin embedding (*Arabidopsis thaliana*). CW, cell wall; G, Golgi stack; M, mitochondrion; T, TGN.

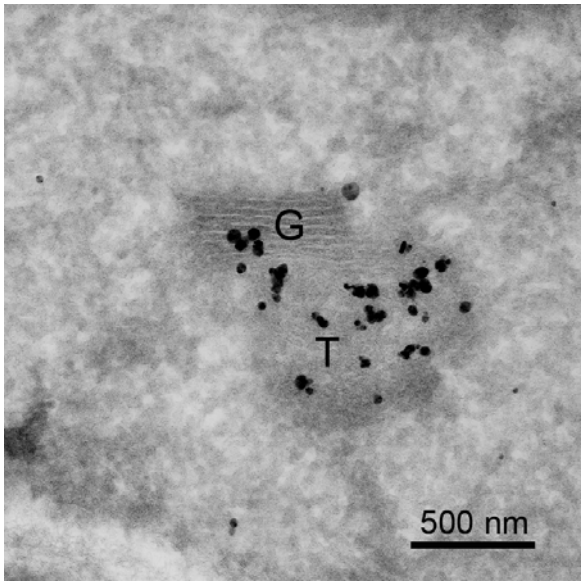

Figure 2. Ultrathin Tokuyasu cryosection of a Golgi stack and associated TGN labelled for ARF1 with silver-enhanced Nanogold. A root tip of an *Arabidopsis* seedling root tip was high-pressure frozen, freeze-substituted, rehydrated and further processed for cryosection labelling. G, Golgi stack; T, TGN.

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