

# III.1 Imaging the Early Secretory Pathway in BY-2 Cells

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## 1 The Early Secretory Pathway in Plants: A Brief Introduction

Although the essential components of the COPI- and COPII-vesiculation machineries are expressed in plants (Andreeva et al. 1998; Jürgens 2004), the early secretory pathway in plants is organized in a much different way to that of animal and yeast cells (Nebenführ et al. 2002; Pavelka and Robinson 2003). This is undoubtedly a consequence of the different physiology of the plant cell, whereby the plant Golgi apparatus has been likened to a polysaccharide factory (Nebenführ and Staehelin 2001). Despite some controversy over several aspects of ER-to-Golgi transport (see below), it can be said that there is widespread agreement over the following key features:

- The plant Golgi apparatus is polydisperse, consisting of discrete Golgi stacks distributed throughout the cytoplasm (Staehelin and Moore 1995).
- The plant Golgi apparatus itself is mobile, and this is microfilament-dependent (Staehelin and Moore 1995).
- In higher plant cells, transitional ER (i. e. specific domains of ER export) does not seem to exist (Pavelka and Robinson 2003).
- Transport between the ER and the Golgi apparatus does not involve a mobile, microtubule-dependent intermediate (ERGIC) compartment (Neumann et al. 2003).
- COPI vesicles only form at the periphery of Golgi cisternae (Pimpl and Denecke 2000).
- The plant Golgi apparatus does not fragment during mitosis (Nebenführ et al. 2000).
- COPII proteins are present in plants and are essential for ER export (Phillipson et al. 2001).

COPI and COPII vesicles were discovered on the basis of vesicle budding assays performed *in vitro* with subcellular fractions enriched in Golgi or ER membranes respectively (Schekman and Orci 1996; Balch 2004). Although the essentiality of the COPI and COPII coat protein recruiting machineries for successful protein transport through the early secretory pathway is generally accepted (see Bonifacino and Glick (2004) and Lee et al. (2004) for reviews),

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the actual existence of these vesicles *in vivo* is still questioned by some researchers in the animal (e.g. Polishchuk et al. 2003) and plant (e.g. Hawes and Satiat-Jeunemaitre 2005) fields. Moreover, although implicit in the “secretory unit model” for ER-Golgi transport (Neumann et al. 2003), one is troubled by the low frequency in which direct tubular contacts between the ER and the Golgi apparatus have been reported in plant cells. It is also difficult to understand why only anterograde but not retrograde transport should be mediated by tubules.

## 2 General Description of the BY-2 Endomembrane System

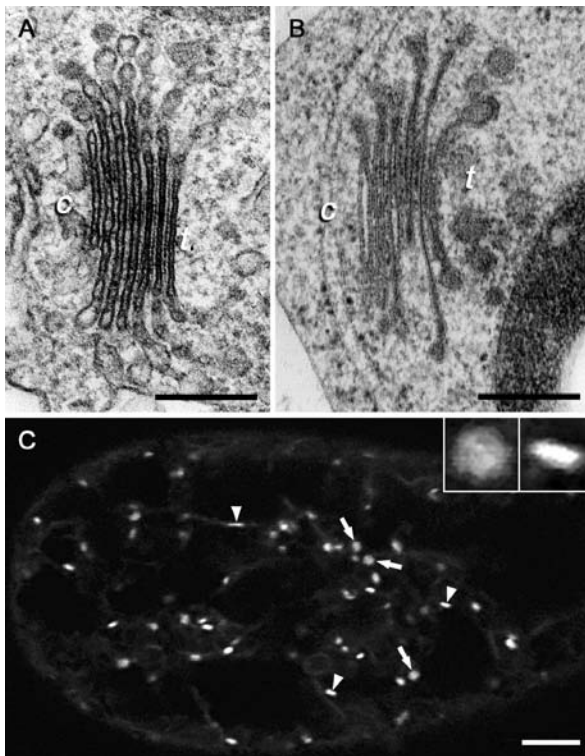
The function of the endomembrane system of tobacco BY-2 cells is essentially that of processing, sorting, and transport of glycoproteins and non-cellulose matrix polysaccharides to the cell wall and the vacuolar compartment(s). Correct targeting and the maintenance of the secretory pathway relies on different proteins, such as chaperones, components of the vesicular transport machinery and the cytoskeleton. The ER is the first compartment of the secretory pathway and also the largest, since it can represent up to 50% of the total cellular membrane surface area (Staehelin 1997; Hara-Nishimura et al. 2004). It is the most versatile organelle, as easily seen in BY-2 cells expressing ER-targeted GFP markers. Thus, the ER is very dense in actively growing cells (3- to 4-day-old cultures), and adopts a very specific organization during mitosis and cytokinesis (Nebenführ et al. 2000; C. Ritzenthaler, unpublished results). As cells become older (6- to 7-day-old cultures) and progressively deprived of carbon sources and oxygen, the ER develops a much looser conformation (C. Ritzenthaler, unpublished results; see also Fig. 3A,B) similar to that observed in mature tobacco epidermal cells (e.g. Fig. 2 in Brandizzi et al. 2002).

The other compartments, further downstream of the ER, are proportionally less affected by growth or environmental conditions, at least at the light microscopical level. Thus, Golgi fluorescent markers show little modification upon ageing of the cells (C. Ritzenthaler, unpublished results) or upon shifting to extreme temperatures such as 40 or 4 °C (D. Robinson and C. Ritzenthaler, unpublished results). The recently characterized prevacuolar compartment (PVC) exhibits in fluorescence microscopy a mobile punctate pattern similar to those observed for Golgi stacks (Tse et al. 2004). By electron microscopy, the prevacuolar marker protein VSR (vacuolar sorting receptor) localizes to multivesicular bodies (Tse et al. 2004). More recently, evidence was provided suggesting that recycling of the VSR from the prevacuolar compartment to the Golgi apparatus is an essential process that is saturable and wortmannin sensitive (daSilva et al. 2005). The vacuoles and the cell wall constitute the final compartments of the secretory pathway. These compartments have been well characterized and BY-2 cells expressing specific markers of these compartments have been obtained (e.g. Mitsuhashi et al. 2000; Czempinski et al. 2002;

Kutsuna and Hasezawa 2002; Kutsuna et al. 2003; Hoffmann and Nebenführ 2004; Yamada et al. 2005).

### 3 The Golgi Apparatus: Structure, Motility and Behaviour During Mitosis

Logarithmically growing BY-2 cells have a Golgi apparatus that is typical for undifferentiated plant cells. It is difficult to ascertain the average number of Golgi stacks per interphase cell, but these were estimated to reach several hundred (Nebenführ et al. 1999). There is no preferential location: Golgi stacks are found in the cortical cytoplasm, in the cytoplasm around the nucleus, and



**Fig. 1.** Golgi visualization in tobacco BY-2 cells. **A** A typical Golgi stack, showing polarity parameters (lumen width, staining, intercisternal filaments) in a clear *cis* (*c*) to *trans* (*t*) gradient; chemical fixation. **B** The same as for **A** but from a high pressure frozen sample (courtesy of Dr. Andreas Nebenführ). **C** Single optical section through the cortex of a cell expressing GmManI-GFP. Arrows and arrowheads point to individual Golgi stacks in face and side-views, respectively. At high magnification, face-viewed Golgi stacks appear as disks (*inset left*), whereas those viewed from the side form a line (*inset right*). Bar 0.25  $\mu\text{m}$  for **A** and **B** and 5  $\mu\text{m}$  for **C**

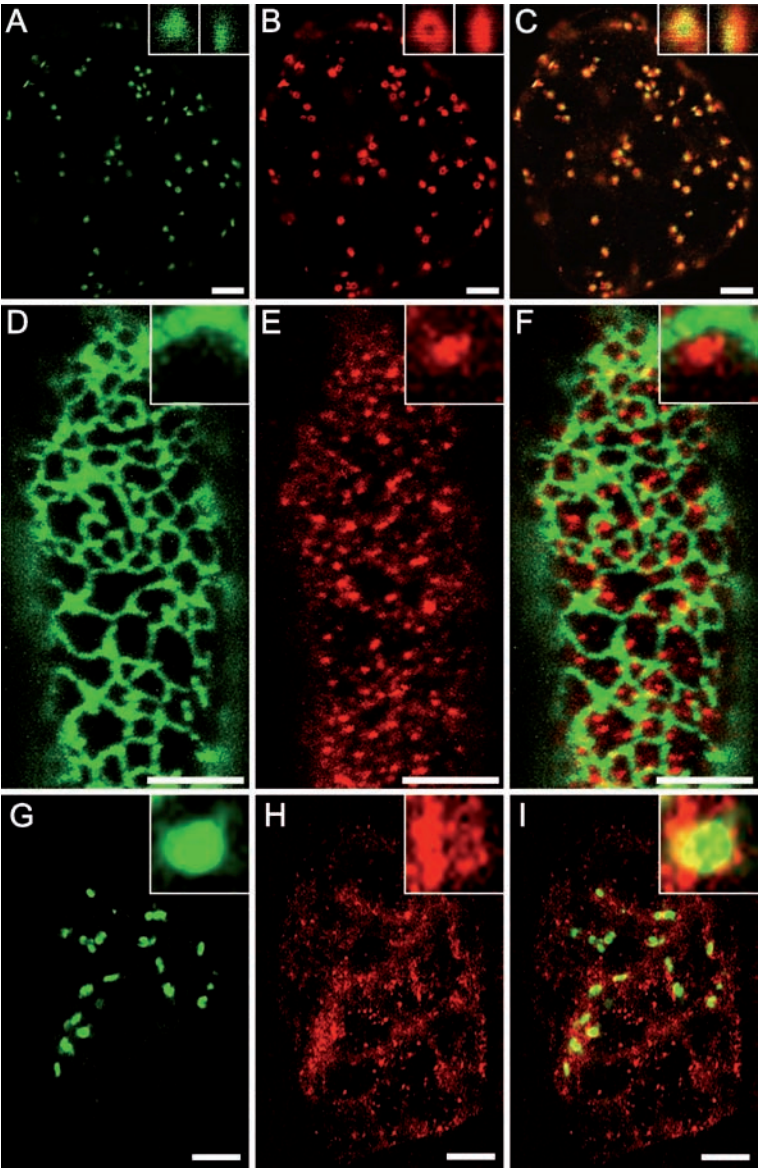
in the transvacuolar strands (Nebenführ et al. 1999). Each stack has between five and eight cisternae, which reveal nicely the classical polarity parameters (see Robinson and Kristen 1982 for details) of luminal width (decreasing *cis* to *trans*), staining intensity (increasing *cis* to *trans*), and intercisternal filaments (medial and *trans*). These features can be seen in both chemically fixed and high pressure frozen fixed cells (Nebenführ et al. 1999; Ritzenthaler et al. 2002; Tse et al. 2004; see also Figs. 1A,B and 3C). It has been reported that Golgi stacks in transgenic cell lines expressing the processing enzyme GmManI (see below) are smaller in both cisternal number and cisternal diameter (Nebenführ et al. 1999).

Our knowledge on the distribution and motility of BY-2 Golgi stacks has been dependent upon the availability of cell lines stably expressing fluorescent Golgi-localized markers. To date, only few (X)FP-labelled enzymes have been successfully used: (1) a GFP- or RFP-tagged class I  $\alpha$ -1,2-mannosidase from *Glycine max* (Nebenführ et al. 1999; Ritzenthaler et al. 2002; Yang et al. 2005). This is an N-glycoprotein processing enzyme, which is located in the *cis* cisternae (Nebenführ et al. 1999); (2) fluorescent Golgi-localized marker a YFP-tagged GONST1 from *A. thaliana* (Tse et al. 2004). This is a sugar nucleotide transporter located in the *trans* cisternae (Baldwin et al. 2001); (3) a GFP- or RFP-tagged sialyl transferase (Boevink et al. 1998; Saint-Jore et al. 2002). This is a terminal glycosyl transferase from mammalian sources, which correctly targets to the *trans* cisternae in plants (Boevink et al. 1998; Saint-Jore et al. 2002); (4) N-glycan GFP-tagged xylosyltransferase. This enzyme is preferentially located in medial cisternae (Follet-Gueye et al. 2003). Corresponding to the orientation of the stack being observed, all of these markers give rise to a fluorescent image that is either punctate (top-view) or rod-like (side view; see Fig. 1C).

In addition to the expression of (X)FP-constructs as a means of visualizing the Golgi apparatus in living BY-2 cells, a specific labelling of Golgi stacks has

► **Fig. 2.** Confocal laser scanning micrographs showing the localization of COPI and COPII components in GFP-HDEL and ManI:GFP transgenic tobacco BY-2 cells. A–C Optical section through the cortical cytoplasm of stably transformed cells expressing the Golgi marker GmMan1-GFP (*green channel*) after fixation and immunolocalization with anti-AtArf1 antibodies (*red channel*). As can be seen in the merged image, the COPI vesicle subunit co-localizes with GFP-fluorescence and is restricted to the margins of the Golgi stacks. *Insets* represent single immunolabelled Golgi stacks under face (*left*) and side (*right*) views at high magnification (five times compared to main image). D–F Optical section through the cortical cytoplasm of stably transformed cells expressing the ER marker GFP-HDEL (*green channel*) after fixation and immunolocalization with AtSec23 antibodies (*red channel*). As can be seen in the merged image and with more detail in the *insets* (magnified five times compared to main images), the COPII vesicle subunit labelling is located either directly on the membrane of the cortical ER or closely adjacent. G–I Optical section through the cortical cytoplasm of a live cell co-expressing the Golgi marker GmMan1-RFP (arbitrarily shown in *green*) and the COPII marker Sec13:GFP (*red channel*). As can be seen in the merged image and in more detail in the *insets* (magnified five times compared to main images), the COPII vesicle subunit marker is dispersed throughout the cortical cytoplasm, forming small punctae that greatly outnumber individual Golgi stacks. Remarkably, COPII labelling is frequently enriched around individual Golgi stacks. Bars 5  $\mu$ m

also been obtained by immunostaining with COPI antibodies (Ritzenthaler et al. 2002). Both AtArf1 and AtSec21 ( $\gamma$ -COP) antibodies bind to the rims of the cisternae, giving rise to a doughnut-like image (see Fig. 2A–C). When the immunostaining is performed on the ManI-GFP cell line, the punctate GFP signal is usually found in the middle of the red immunofluorescence signal (Fig. 2A–C). The immunostaining pattern reflects COPI-vesicle formation which takes place at the tubular periphery of the cisternae, a feature confirmed



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