Studying Golgi protein dynamics after disruption of Golgi membranes in tobacco

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The plant Golgi apparatus is an extremely versatile and dynamic structure that is often closely associated with the membranes of the endoplasmic reticulum (ER) [1]. Both organelles form a secretory unit [2], which may be tethered together by a number of golgins that might also be part of a Golgi matrix [3]. The plant Golgi complex has a striking morphology as it consists of numerous distinct stacks of flattened cisternae displaying a *cis*-to-*trans* polarity [4]. Within a Golgi stack, N-glycan processing enzymes are arranged in a highly ordered fashion and thereby form an assembly line that facilitates the step-by-step modification of oligosaccharides on glycoproteins [5,6]. Thus, these enzymes provide excellent tools to investigate antero- and retrograde transport processes between the ER and Golgi apparatus.

In tobacco, confocal microscopy studies have indicated that three transiently expressed plant N-glycan processing enzymes [6,7,8] tagged with various fluorescent proteins occur in distinct but overlapping compartments of the Golgi apparatus. In order to study the structural characteristics and organisation of the plant Golgi apparatus in more detail, we first deconstructed the whole complex by inducing the reabsorption of Golgi membranes into the ER, and afterwards monitored Golgi stack reassembly. One approach was to treat tobacco leaves with the secretory inhibitor Brefeldin A (BFA). However, a more controlled reabsorption of Golgi membranes was achieved by using an inducible system for the expression of the GTP-locked form of the small GTPase Sar1 [9], which is responsible for the initiation of the COPII coat at ER export sites. After either BFA or Sar1-GTP-induced disassembly of Golgi stacks, the first event of Golgi membrane transport to the ER unexpectedly was the loss of *trans*-Golgi associated golgins followed by *trans*-located glycosyltransferases. Golgi disassembly continued in a *trans*-to-*cis* direction. Finally, *cis*-located Golgi matrix proteins were redistributed into the ER or cytoplasm respectively, but were also observed in punctate remnant structures.

On rebuilding of Golgi stacks during BFA washout, *cis*-located Golgi matrix proteins assembled on Golgi membranes prior to Golgi enzymes that reside in the *cis*-half of the Golgi stack. Medial and *trans*-proteins followed sequentially.

Our data show that in tobacco leaves the disassembly of Golgi stacks starts at the *trans*-side and continues towards the *cis*-Golgi cisternae. The formation of new Golgi cisternae occurs in a *cis*-to-*trans* direction with matrix proteins assembling on Golgi membranes prior to Golgi-resident enzymes. We suspect that a *cis*-Golgi matrix may serve as a template structure for the formation of new Golgi stacks.

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