Golgi Matrix Proteins in Plants

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Introduction

At the centre of the cell's secretory pathway lies the Golgi apparatus, a collection of stacks of membrane cisternae. Whereas in animals the stacks are organised end to end into a larger ribbon structure, plant Golgi stacks are mobile bodies that are spread throughout the cytoplasm. The Golgi apparatus receives proteins from the endoplasmic reticulum (ER) and processes them further (glycosylation) before they are packaged into vesicles for transport to the cell surface or to the vacuole.

In animals and yeast, a number of **Golgi Matrix Proteins** have been described that are believed to serve as structural support for the cisternae and to play a role in tethering of vesicles to target membranes. They are large (80 – 400 kD) proteins with extended coiled-coil domains. This work describes the identification of the first plant GMPs.

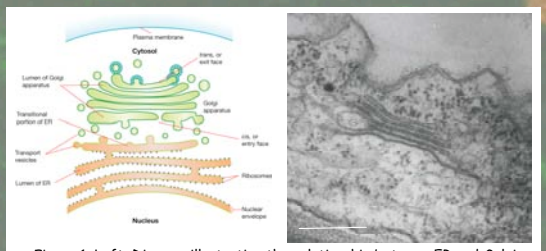

Figure 1: Left: Diagram illustrating the relationship between ER and Golgi apparatus. Vesicle transport to and from the Golgi stack is shown. Right: electron micrograph of a Golgi stack in a potato tuber parenchyma cell, post-fixed with zinc iodide and osmium tetroxide. Bar = 300 nm (courtesy of Trudi Gillespie)

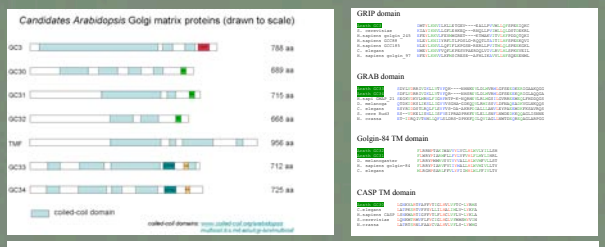

Figure 2: Left: schematic representation of AtGMP domain structure. Right: Alignments of GRIP and GRAB domains and GMP transmembrane domains

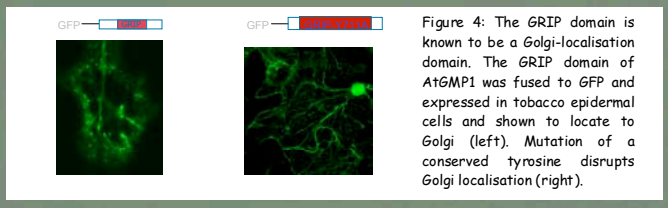

Figure 4: The GRIP domain is known to be a Golgi-localisation domain. The GRIP domain of AtGMP1 was fused to GFP and expressed in tobacco epidermal cells and shown to locate to Golgi (left). Mutation of a conserved tyrosine disrupts Golgi localisation (right).

Results

An extensive collection of *Arabidopsis* coiled-coil proteins (www.coiled-coil.org/arabidopsis/) was searched for proteins with homology to animal or yeast Golgi Matrix Proteins using the BLAST algorithm. A number of *Arabidopsis* proteins were identified showing homology to animal GMPs. The regions of homology were not in the coiled-coil domains but in transmembrane domains and in two Golgi localisation domains called GRIP domain and GRAB domain (Figure 2). The GRIP domain was fused to GFP and shown to localise to Golgi stacks (Figure 4).

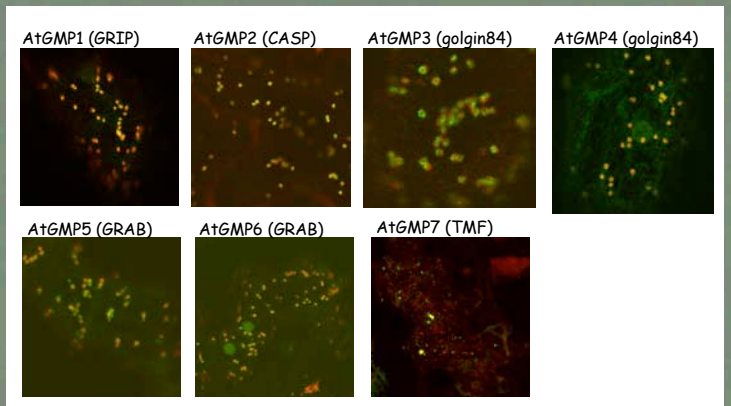

Figure 3: GFP fusion proteins of *Arabidopsis* Golgi Matrix Proteins (GMPs) were expressed in a tobacco line expressing the Golgi marker sialyl transferase (st)-mRFP (red) using agro-infiltration. Epidermal cells were imaged by confocal microscopy at 2 dpi.

Seven of the *Arabidopsis* coiled-coil domain proteins co-localised with the Golgi marker sialyl transferase (st)-mRFP (Figure 3). They are therefore named AtGMP1 – AtGMP7. AtGMP1 shows polar Golgi localisation. AtGMP2 – AtGMP4 form rings around the Golgi stacks. AtGMP7 stains dots that over time associate and dissociate with Golgi stacks, reminiscent of the Trans Golgi Network (TGN).

The fungal toxin Brefeldin A is an inhibitor of the secretory pathway. It is known to disrupt Golgi stacks, leading to absorption of many Golgi proteins back into the ER. Some Golgi proteins have been reported to accumulate in small bodies, so-called 'BFA compartments'. It is hypothesized that these proteins originate from the *trans* Golgi (Ritzenthaler et al. 2002). BFA treatment led to re-localisation of AtGMP2 and AtGMP4 into the ER. AtGMP1, AtGMP3, AtGMP5 and AtGMP6 were detected on bodies, possibly BFA compartments, suggesting that they are located in the *trans* Golgi (Figure 5).

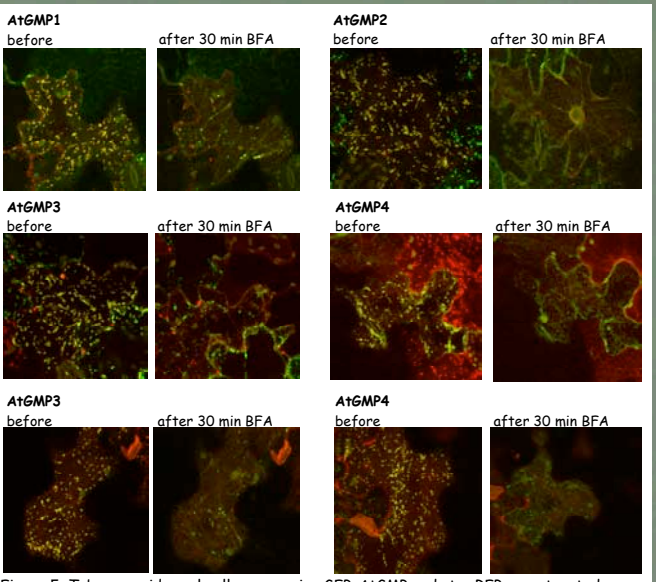

Figure 5: Tobacco epidermal cells expressing GFP-AtGMP and st-mRFP were treated with Brefeldin A (10 _g/ml). Cells were imaged before and after treatment (ca. 30 min).

Conclusion

Seven plant proteins with homology to animal Golgi matrix proteins were identified and localised to Golgi stacks in tobacco cells. In future work we will localise the proteins to Golgi subcompartments by confocal and electron microscopy. We will also focus on functional analysis of the AtGMPs

Ritzenthaler et al., (2002), Plant Cell **14** (237-261)