In search of plant Golgi matrix proteins

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In our modern world, we rely on the efficient transport of goods for our everyday needs. Road blocks that stop traffic between cities turn the country into chaos and our lives upside down. Just as in our own society, transport of materials from one location in the plant cell to another is absolutely essential for normal cell functions.

Every single protein that is synthesised within the cytoplasm needs to find its way to the location where it will carry out its function. The information that tells a protein where to go is often encoded in the shape of that protein. In plant cells, proteins may be 'addressed' to the outside of the cell (secretion), to protein bodies (for storage) or to various organelles such as the vacuole, chloroplasts, mitochondria and peroxisomes. For many of these proteins, the journey to their final destinations starts at the endoplasmic reticulum (ER), a three dimensional network of tubules that forms the basis of the protein sorting machinery. The address label ('post code') of this class of proteins is a small sequence at the beginning of the protein called a signal peptide. Each newly synthesised protein that contains a signal peptide is directly guided into the ER. Extensive sorting of the proteins in the ER results in the selection of a subset that is destined for the extracellular space or the vacuoles. This subset of proteins is sent to a second major processing centre, the Golgi apparatus.

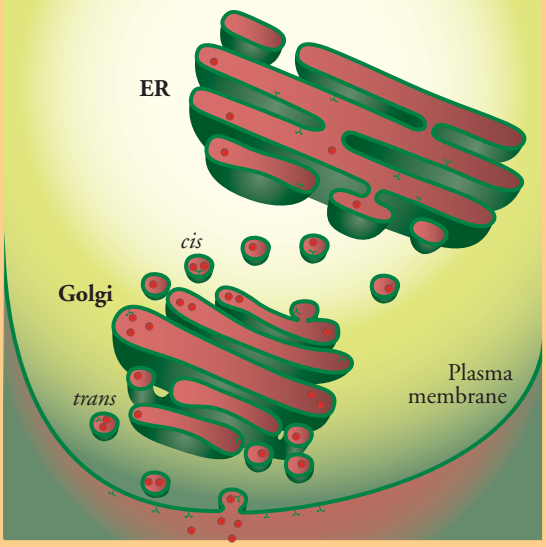

Figure 1 Diagram illustrating the relationship between the ER and the Golgi apparatus. Vesicle movement is shown transporting proteins from the ER to the Golgi stack and from the Golgi to the plasma membrane.

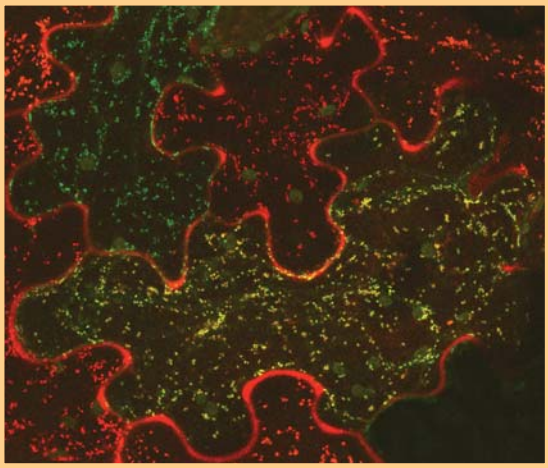

Figure 2 *Nicotiana tabacum* epidermal cells expressing the *trans*-membrane domain of sialyl-transferase fused to the red-fluorescent protein (ST-mRFP; a Golgi marker) and AtGRIP fused to the green-fluorescent protein (GFP). Some cells express only ST-mRFP or the AtGRIP-GFP fusion protein, resulting in cells with either purely red or purely green Golgi stacks. The cell in the lower centre of the picture expresses both markers. Co-localisation of the red and the green fluorescent proteins appears yellow.

The Golgi apparatus consists of stacks of cisternae that are like large, flat vesicles. One of the functions of the Golgi apparatus is the glycosylation of proteins (modification by addition of sugar groups). The Golgi cisternae contain the enzymes that catalyse this glycosylation. Proteins enter the Golgi on the *cis* side and travel through the Golgi until they reach the *trans* side. Transport to and from the cisternae is carried out by means of small vesicles that pinch off from one cisterna and fuse to the next. From the *trans* Golgi, the proteins are sent within vesicles to their destinations, the plasma membrane or the vacuole. In animal cells, the Golgi stacks aggregate around the nucleus. They disintegrate during mitosis and reform afterwards. In contrast, in plants the Golgi stacks are dispersed throughout the cytosol and move with or along the ER. They remain intact throughout mitosis. The molecular basis and the purpose of the differences

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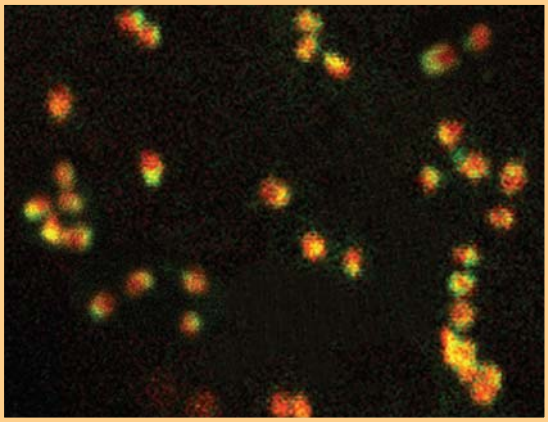

Figure 3 Magnification of individual Golgi stacks with both the red Golgi marker (ST-mRFP) and the green fluorescing AtGRIP-GFP. Note that the red marker labels the entire Golgi stack and shows them as round bodies whereas the green AtGRIP locates to one end of each stack.

between animal and plant Golgi is unknown and is an intriguing question that we are trying to address.

In the last few years, a large number of proteins from animal Golgi stacks have been characterised that give structural support to the Golgi. They are involved in the stacking of cisternae and in binding (tethering) of vesicles to their destination membranes in the Golgi. These proteins are called **Golgi matrix proteins** and are found to be components of a proteinaceous matrix linking Golgi cisternae together. This matrix had already been noticed in electron microscopy studies. The Golgi matrix proteins are generally large proteins containing long coiled-coil domains that form rod-like structures. These domains are commonly found in structural proteins.

Although plant Golgi were also found to have a proteinaceous matrix around them, its composition is unknown, as is how the Golgi stacks are held together as they move around the cell. To find plant Golgi matrix proteins, we used the BLAST programme to compare animal Golgi matrix proteins to the protein sequences from the model plant *Arabidopsis thaliana.* In this way, an *Arabidopsis* protein was identified possessing a feature called a 'GRIP domain'. This protein domain has been found in a number of animal and yeast Golgi matrix proteins. Apart from the GRIP domain, the *Arabidopsis* protein shows extensive coiled-coil regions. The protein was christened AtGRIP (*Arabidopsis thaliana* GRIP domain protein). We expressed AtGRIP as a fusion with the Green Fluorescent Protein (GFP) in tobacco epidermal cells. The tobacco line used for this study stably expresses a red fluorescent marker protein in Golgi stacks. When observed using a fluorescence or confocal laser-scanning microscope (CLSM), the Golgi stacks light up in a bright red colour. In epidermal cells expressing both the red Golgi marker and the AtGRIP-GFP fusion, Golgi stacks contained both red and green fluorescence. When we magnified individual Golgi stacks, we noticed that the AtGRIP-GFP fusion protein was located to one end of the Golgi stack, forming a sort of cap on the round, red dots. This clearly shows that AtGRIP is a Golgi protein. Judging from its structure and domains, we can confidently say that it is a Golgi matrix protein, the first plant Golgi matrix protein to be discovered.

If plant cells are treated with a drug called Brefeldin A (BFA), Golgi stacks disintegrate instantly. Most Golgi proteins are retrieved into the ER. However, it has been shown that proteins residing on the *trans* side of the Golgi do not go back into the ER but instead aggregate into bodies that move in the cytoplasm. This is exactly what happened to the GFP-labelled AtGRIP when the cells were treated with BFA. The green fluorescence aggregated into spots that were clearly larger than Golgi stacks, and the aggregates moved throughout the cell. This suggests that AtGRIP is located on the *trans* side of the Golgi.

Further research will focus on elucidating the role of AtGRIP in protein trafficking, and trying to identify new proteins that interact with AtGRIP. In addition, we are continuing our search for other plant Golgi matrix proteins. All of this is aimed at obtaining a better understanding of how the plant Golgi apparatus controls protein trafficking and secretion in plant cells.