

In vivo interactions of higher plant Golgi matrix proteins by fluorescence lifetime imaging

C. R. Hawes and A. Osterrieder

School of Life Sciences, Oxford Brookes University,
Oxford, OX3 0BP, UK

S. W. Botchway and C. Stubbs

Central Laser Facility, STFC, Rutherford Appleton Laboratory,
Harwell Science & Innovation Campus, Didcot, OX11 0QX, UK

Main contact email address

chawes@brookes.ac.uk

Introduction

The Plant Endomembrane Group at Oxford Brookes University researches into the structure and function of the higher plant secretory pathway which is responsible for the synthesis and processing of proteins, lipids and carbohydrates that are either going to be stored in the cell or secreted to the external environment. The Golgi apparatus, the central organelle of this pathway, consists of a series of stacks of small flattened membrane sacs termed cisternae with a polarised structure. Here proteins are modified and carbohydrates synthesised. These stacks are motile within the cell and in leaves stay associated with another compartment upstream of the production process, the endoplasmic reticulum (ER).

It has long been known that the structure of the plant Golgi apparatus in higher plants is generally different to that in mammalian and fungal systems, comprising many discrete stacks of cisternae (or Golgi bodies) that appear more or less randomly distributed throughout the cytoplasm (Hawes 2005)^[1]. However, it has taken the development of live cell imaging of the plant secretory pathway to reveal the unique nature of the plant Golgi. Previous work from the Brookes laboratory has demonstrated that the individual Golgi bodies are motile and traffic, in an actin dependent manner, in the cytosol (Boevink *et al.* 1998)^[2]. In many tissues there is a close relationship between the Golgi and the endoplasmic reticulum (ER) and we have proposed that in leaf cells the Golgi moves over or even with the ER membrane, whilst complexed to ER exit sites where cargo transfer takes place. Both pharmacological studies and a photobleaching strategy have demonstrated that cargo transfer is energy dependent but does not require active participation of the cytoskeleton (Saint-Jore *et al.* 2002^[3], Brandizzi *et al.* 2002^[4], daSilva *et al.* 2005^[5]). To date we have no evidence as to whether this close association of the Golgi to the ER is due to direct membrane continuity or whether specific proteins form tethering complexes which are responsible for linking the two organelles together.

One major advance in our understanding of the Golgi complex in mammalian and yeast cells has been the discovery of a family of proteins called the golgins which are important in maintaining the integrity of the Golgi apparatus. Golgins are large proteins with coiled-coil domains which consist of heptad repeats, forming amphipathic α -helices that twist into a super-coil forming long rod-like structures (Barr & Short 2003^[6], Gillingham & Munro 2003^[7]). It has also been shown that interaction of Golgins with specific small GTPase enzymes that are required to recruit the proteins to the Golgi (Latijnhouwers *et al.* 2005a^[8]). A number of different roles have been assigned to the golgins in recent years. For instance it was shown that the mammalian golgins GM130, p115 and

Giantin interact to form a Golgi skeleton that remains in the cytoplasm after dissolution of the Golgi membranes with Brefeldin A (Seemann *et al.* 2000^[9]). This skeleton may also form a template for reformation of the Golgi ribbon after dispersal during nuclear division. It is also thought that these three proteins form a complex required for maintenance of the Golgi compartments nearest to the ER. Depletion of another Golgin, Golgin-84 results in the fragmentation of the mammalian Golgi ribbon into separate stacks (Diao *et al.* 2003^[10]).

Over the past three years we have identified and cloned a number of plant Golgins and located them to the Golgi bodies in tobacco leaf epidermal cells using fluorescent protein constructs (Latijnhouwers *et al.* 2005b^[11]). Biochemically we have shown that some of these proteins interact with regulatory GTPases and with each other. The objective of this current work was to investigate the feasibility of measuring interactions between these Golgi proteins and with regulatory GTPases *in vivo*, within the 1 μ m diameter Golgi bodies in tobacco leaf epidermal cells.

To determine whether any two chosen proteins under investigation were assembling or interacting, time-resolved fluorescence (Förster) resonance energy transfer (FRET) was used. The occurrence of FRET between constructs was established using fluorescence lifetime imaging (FLIM). That is measuring reduction in fluorescence lifetime of the donor fluorophore on transfer of non-radiative energy to the acceptor.

Results and discussion

A range of Golgi matrix proteins and small regulatory GTPases were transiently expressed in tobacco leaves using the agrobacterium infiltration techniques (Sparkes *et al.* 2006)^[12] either alone as green fluorescent protein (GFP) tagged constructs, or in pairs as GFP and monomeric red fluorescent protein (mRFP) tagged constructs. This pairing was chosen for FRET as the emission signal of GFP overlaps significantly with the excitation wavelengths of mRFP which is necessary for FRET. Imaging of expression in leaf tissue was carried out with a Nikon eC1 confocal attached to a TE2000U microscope (Fig. 1) and lifetime

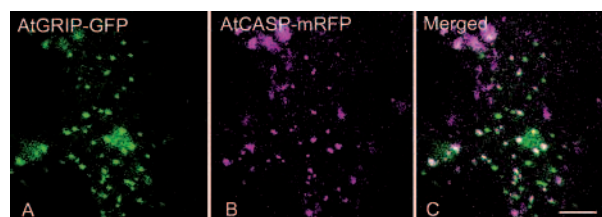


Figure 1. Labelling of tobacco leaf Golgi with AtGRIP-GFP (A) and mRFP-AtCASP (B). The merged image shows colocalisation of the two proteins (C). Scale bar = 10 μ m.

imaging carried out on a 2-photon microscope setup. Laser light at a wavelength of 700-1000 nm was obtained from a Titanium Sapphire (Ti:Sa, Mira), 75 MHz, mode-locked laser (8W 532 nm, Verdi pumped, Coherent Lasers), with a pulse width of 180 fs. Analysis of FLIM images were performed using SPC Image 2.0 software (Becker & Hickl).

The fluorescence lifetime of GFP was obtained from images of expression of Golgin constructs in tobacco leaf epidermal cells and was in the range of 2.4 – 2.6 ns (Fig. 2a). As a control a double expression was carried out with GFP and mRFP fused to the same Golgi protein, the ST marker (Boevink *et al.* 1998)^[2]. This resulted in double labelling of all Golgi in transformed cells and lifetime images of GFP in these cells showed no reduction compared to a single expressed GFP construct. This confirms that simply positioning two fluorochromes in the same location in a Golgi membrane does not result in FRET as no specific protein/protein interaction is taking place. We next expressed two Golgins, AtGRIP-GFP (Fig. 1a) and mRFP-AtCASP (Fig. 1b), which are predicted to target the *trans*- and *cis*-faces of the Golgi respectively. Confocal imaging showed them to colocalise on the Golgi (Fig. 1c), yet FLIM imaging showed no quenching of the GFP lifetime and thus no FRET (Fig. 2b). However, upon co-expression of AtGRIP-GFP with an mRFP fusion of the GTPase, ARL1 which is predicted to regulate the recruitment of AtGRIP onto the *trans*-Golgi, a significant reduction in fluorescence lifetime was recorded as seen on the pseudocoloured lifetime image (Fig. 2c). This result confirms the *in vitro* data that we have obtained from yeast two-hybrid assays and GST pull-downs with these two proteins (Latijnhouwers *et al.* 2005a^[8]). Similar experiments showed no interaction between the c-terminal domain of the *trans*-Golgi matrix protein TMF and the GTPase RabH1b, but interaction between RabH1b and AtGRIP.

Conclusions

From this work we have demonstrated that it is possible to obtain FRET data using FLIM from organelles within plant cells with a diameter as small as 1 μm . Data from numerous Golgi stacks can be obtained from a single FLIM image (Fig. 2) and therefore statistically valid sampling should be relatively easily. Future work will ascertain the interactions of a range of Golgi associated proteins including transferases, the membrane fusion proteins SNAREs and other Golgins. It should then prove possible to measure the progress of these protein interactions during stages of Golgi biogenesis.

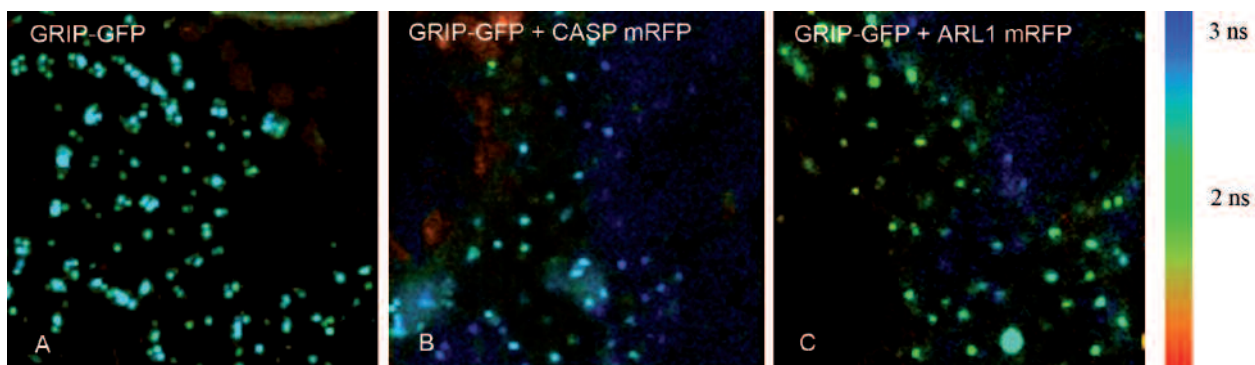


Figure 2. Pseudo-coloured fluorescence lifetime images of: AtGRIP-GFP alone (A, average lifetime 2.4 ns), AtGRIP-GFP plus mRFP-AtCASP showing no GFP quenching (B, average lifetime 2.4 ns), AtGRIP-GFP plus ARL1-mRFP showing quenching of GFP (C, average lifetime 2 ns).

Acknowledgements

Work on plant Golgins was funded by a BBSRC grant to CH.

References

1. C. R. Hawes, *New Phytologist*. **165**, 29-44 (2005).
2. P. Boevink, K. Oparka, S. Santa Cruz, B. Martin, A. Betteridge and C. Hawes, *Plant J* **15**, 441-447 (1998).
3. C. M. Saint-Jore, J. Evins, H. Batoko, F. Brandizzi, I. Moore and C. Hawes, *Plant J* **29**, 661-678 (2002).
4. F. Brandizzi, E. L. Snapp, A. G. Roberts, J. Lippincott-Schwartz and C. Hawes, *Plant Cell* **14**, 1293-1309 (2002).
5. L. L. P daSilva, E. L. Snapp, J. Denecke, L. Lippincott-Schwartz, C. Hawes and F. Brandizzi, *Plant Cell* **16**, 1753-1771 (2004).
6. F. A. Barr and B. Short, *Curr. Op. Cell Biol.* **15**, 405-413 (2003).
7. A. K. Gillingham and S. Munro, *Biochim Biophys Acta* **1641**, 71-85 (2003).
8. M. Latijnhouwers, C. Hawes, C. Carvalho, K. Oparka, A. K. Gillingham and P. Boevink, *Plant Journal* **44**, 459-470 (2005a).
9. J. Seemann, E. Jokitalo, M. Pypaert and G. Warren, *Nature* **407**, 1022-1026 (2000).
10. A. Diao, D. Rahman, D. J. Pappin, J. Lucocq and M. Lowe, *J Cell Biol* **160**, 201-212 (2003).
11. M. Latijnhouwers, C. Hawes and C. Carvalho, *Current Opinion in Plant Biology* **8**, 632-639 (2005b).
12. I. Sparkes, J. Runions, A. Kearns and C. Hawes, *Nature Protocols* **1**, 2019-2025 (2006).